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FORMULATION OF A PREBIOTIC PLANT-BASED PROTEIN RICH ICE CREAM USING GERMINATED AMARANTH MILK, FOXNUT AND TRAGACANTH GUM

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Nims University Rajasthan, Jaipur

2026

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***Dissertation Submitted to the Nims University Rajasthan, Jaipur for the Degree of***

**Master of Science**

**in**

**Nutrition & Dietetics**

***Submitted by***

**DRISHTI LALWANI**

**(Enrollment No.145634)**

***Under the Supervision of***

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**Department of Nutrition & Dietetics**
**Nims College of Allied & Healthcare Sciences**
**Nims University Rajasthan, Jaipur**
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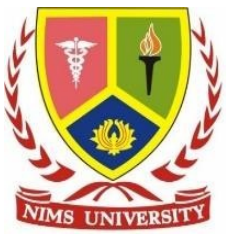



**Department of Nutrition & Dietetics**

**Nims College of Allied & Healthcare Sciences**

**Nims University Rajasthan, Jaipur**


**2025**

**Nims University Rajasthan, Jaipur**

Date:.....

# CERTIFICATE OF ORIGINALITY

The research work embodied in this entitled “Formulation of a Prebiotic Plant-Based Protein Rich Ice Cream Using Germinated Amaranth Milk, Foxnut, and Tragacanth Gum ” has been carried out by me in the Department of Nutrition & Dietetics , Nims University Rajasthan, Jaipur, India. The manuscript has been subjected to plagiarism check by Turnitin Plagiarism detection software. The work submitted for consideration for the award of Master Degree is original.


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## DECLARATION

This is to declare that the present research work entitled "**Formulation of a Prebiotic Plant-Based Protein Rich Ice Cream Using Germinated Amaranth Milk, Foxnut, and Tragacanth Gum**" is an original research work conducted by **Drishti Lalwani** under the supervision of **Dr. Damini Soni,** Department of Nutrition & Dietetics, Nims University Rajasthan, Jaipur. This research work is original to the best of my knowledge and has not been submitted earlier, in part or in full for other degree or diploma in this or any other university.

**DRISHTI LALWANI**

It is hereby, recommended that the thesis may be placed before the examiners for evaluation.

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# Acknowledgment

I thank God Almighty for giving me the strength, good health, and resources to complete this research successfully. I express my sincere gratitude to my guide, **Dr. Damini Soni**, Assistant Professor, Department of Nutrition and Dietetics, NIMS College of Allied and Healthcare Sciences, NIMS University, Rajasthan, Jaipur, for her guidance and support during this study. I also thank the faculty and staff of the department for their assistance throughout the course of this work. I express my deepest and most sincere love and gratitude to my parents, their constant encouragement, emotional strength, and endless patience have seen me through every demanding phase of my research, and I must say that their faith in me has always been my biggest motivation. I take this opportunity to express my sincere appreciation for the invaluable support provided to me by my cousin brother, **Mr. Garv Jivnani**, who dedicated his time, effort, and enthusiasm towards helping me prepare the practical aspects of my ice cream formulations. His contribution to my research work has been invaluable towards the successful completion of my research.I extend my warm and heartfelt thanks to my cousin sister, **Ms. Yashi Jivnani**, for her creative expertise and meticulous efforts in designing the nutritional label and packaging of the developed product. Her aesthetic sensibilities have ensured that the packaging design is not only visually appealing but also fully compliant with the FSSAI regulatory standards. Lastly, I would like to acknowledge with great respect all the various researchers, scientists, and scholars who, through their published work, have formed the academic basis for this work. The various contributions to the field of functional food science, plant-based nutrition, prebiotic science, and vegan food product development have greatly informed, guided, and enriched the academic content of this dissertation. I am grateful to all of them for their

pioneering work in this field. I am grateful to one and all who, in one way or another, have contributed to this research work.

Place: Jaipur,
**Drishti Lalwani** Date:


## ABSTRACT

### Background

The increasing market demand for plant-based functional foods and the increased awareness of gut health have created a major market window for vegan prebiotic high protein-containing frozen desserts.

### Objectives

The present study was initiated with an aim to formulate and characterize a vegan prebiotic high protein ice cream using germinated amaranth milk-based protein, makhana, jackfruit seed powder, gond katira, monk fruit sweetener, FOS/inulin, almond butter, coconut milk, dark chocolate, cocoa powder, cashews, and vanilla extract.

### Methods

The study was conducted at NIMS University, Jaipur, Rajasthan and was carried out in six systematic phases. Four different formulations were developed, one control formulation and three experimental formulations, in which the amounts of gond katira and cashews were varied. Sensory evaluation was performed using a 9-point hedonic scale method with 15 semi-trained panelists. The highest-scoring formulation was selected for further nutritional and physicochemical analysis through a NABL accredited laboratory.

### Results

The formulation containing 45g gond katira and 85g cashews was selected as the best formulation with an overall acceptability score of 6.1 ± 1.7. NABL accredited nutritional analysis confirmed that the product contains 6.87g protein, 290.86 Kcal total energy, 7.90g fat, 51.97g carbohydrates, 1.19g crude fibre, 29.84% moisture, a melting rate of 30 minutes, total phenolic content of 195 mg GAE, DPPH radical scavenging activity of 24.90%, off white colour, and an overrun of 78% per 100g. The cost of production was calculated at ₹424.07 per 500g unit. The product was packed in a food-grade HDPE container, labelled as per FSSAI

Labelling and Display Regulation, 2020, and named Greenz Ice Cream under the brand name Nutrii Mantra.

**Conclusion**

The developed formulation is nutritionally superior, organoleptically acceptable, microbiologically safe, and commercially feasible, with strong potential in the Indian market of functional and plant-based frozen desserts.

**KEY WORDS**

Vegan ice cream, prebiotic, high protein, germinated amaranth milk, makhana, jackfruit seed powder, gond katira, monk fruit sweetener, functional food, plant-based, gut health, antioxidant, FSSAI, Nutrii Mantra.

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## LIST OF ABBREVIATIONS

| Sr. No | ABBREVATIONS | EXPANSION |
|---|---|---|

| | | |
|---|---|---|
| **1** | **AAA** | Formulation Variation T1 (Gond Katira 35 g, Cashews 70 g) |
| **2** | **ABTS** | 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) — antioxidant assay |
| **3** | **ANOVA** | Analysis of Variance |
| **4** | **AOAC** | Association of Official Analytical Chemists |
| **5** | **ATCC** | American Type Culture Collection |
| **6** | **BBB** | Formulation Variation T2 — Most Acceptable (Gond Katira 45 g, Cashews 85 g) |
| **7** | **BMJ** | British Medical Journal |
| **8** | **CAGR** | Compound Annual Growth Rate |
| **9** | **CCC** | Formulation Variation T3 (Gond Katira 55 g, Cashews 55 g) |
| **10** | **CFU** | Colony Forming Units |
| **11** | **CHO** | Carbohydrate |
| **12** | **DDD** | Control Formulation |
| **13** | **DPPH** | 2,2-Diphenyl-1-picrylhydrazyl — free radical scavenging assay |
| **14** | **EAAI** | Essential Amino Acid Index |
| **15** | **FAO** | Food and Agriculture Organization of the United Nations |
| **16** | **FDC** | Food Data Central (USDA) |
| **17** | **FOS** | Fructooligosaccharides |
| **18** | **FRAP** | Ferric Reducing Antioxidant Power |
| **19** | **FSSAI** | Food Safety and Standards Authority of India |

| | | |
|---|---|---|
| **20** | **GAE** | Gallic Acid Equivalents |
| **21** | **GOS** | Galactooligosaccharides |
| **22** | **HDPE** | High-Density Polyethylene |
| **23** | **ICMR** | Indian Council of Medical Research |
| **24** | **IFCT** | Indian Food Composition Table |
| **25** | **IMARC** | International Market Analysis Research and Consulting Group |
| **26** | **ISAPP** | International Scientific Association for Probiotics and Prebiotics |
| **27** | **ISO** | International Organisation for Standardisation |
| **28** | **NABL** | National Accreditation Board for Testing and Calibration Laboratories |
| **29** | **NIMS** | Nims Institute of Medical Sciences |
| **30** | **NIN** | National Institute of Nutrition |
| **31** | **OBMS** | Oat-Based Milk Substitute |
| **32** | **ORAS** | Opal Research and Analytical Services |
| **33** | **PBMA** | Plant-Based Milk Alternatives |
| **34** | **PBMS** | Plant-Based Milk Substitutes |
| **35** | **RDA** | Recommended Dietary Allowance |
| **36** | **RSM** | Response Surface Methodology |
| **37** | **SD** | Standard Deviation |
| **38** | **SMP** | Skimmed Milk Powder |
| **39** | **SOP** | Standard Operating Procedure |
| **40** | **SPSS** | Statistical Package for the Social Sciences |

| | | |
|---|---|---|
| **41** | **SVTL** | SV Testing Laboratories Pvt. Ltd. |
| **42** | **TEAC** | Trolox Equivalent Antioxidant Capacity |
| **43** | **TISTR** | Thailand Institute of Scientific and Technological Research |
| **44** | **TPC** | Total Phenolic Content |
| **45** | **TSS** | Total Soluble Solids |
| **46** | **UPF** | Ultra-Processed Food |
| **47** | **USD** | United States Dollar |
| **48** | **USDA** | United States Department of Agriculture |
| **49** | **WPC** | Whey Protein Concentrate |
| **50** | **XOS** | Xylooligosaccharides |

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# CHAPTER - I

# INTRODUCTION

# INTRODUCTION

**1.1. Ice Cream: Importance, Composition, and Market Evolution**

**1.2. Health Concerns Associated with Conventional Ice Cream**

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## 1.1. ICE CREAM - Importance, Composition, and Market Evolution

Ice cream is really special when it comes to food that is made in factories. It is something that people enjoy eating because it tastes good it is a tradition that many cultures share. It is also a way for scientists to make food that is good for us. People of all ages and backgrounds from around the world eat ice cream. Over time ice cream has changed from a mix of frozen milk, sugar and flavourings to a very complicated food system that people have carefully designed.

When we look at ice cream from a point of view it is made up of many different parts like ice, fat, air and a special liquid that is all mixed together in a way that is not very stable. Ice cream has ice crystals, globules that are stuck together air bubbles and a special liquid part that is not frozen all of which are in a delicate balance, with each other. [1,2] The way these phases are made up. Like how big the ice crystals are,

how much the fat is mixed up how much air is, in the mix. The arrangement of air bubbles is what determines the taste and texture of the ice cream. It gives the ice cream a creamy, smooth, and delicious flavor. It is also responsible for developing the flavor and making the ice cream melt slowly.[3]

An understanding of the microstructure of ice cream is very essential in the formulation, production, and development of ice cream products. In ice cream science and ice cream product development, this knowledge plays a critical role. Some achievements have been recorded by researchers regarding the understanding of these tiny structures. The use of such techniques as X-ray microtomography and cryo scanning electron microscopy has helped accomplish this.[4]

Many types of ingredients are required in the preparation of ice cream. They include milk, or any other plant source sweeteners, which give the ice cream its sweetness and freezing properties. Other ingredients include stabilizers, emulsifiers, flavoring agents, colorants and some additives which have various nutritional values. These ingredients have some influence on ice creams. They not only give it sweetness but also its freezing, consistency, and melting properties.[1] Stabilizers and emulsifiers act jointly to help regulate rheology and retain the stability of air bubbles in ice cream. This has an impact, first of all, on the texture and melting properties of the product. It is necessary to note that stabilizers and emulsifiers are extremely important components as they guarantee proper consistency and melting characteristics of ice creams. [5] Modern expectations of consumers about ice cream have completely changed. Nowadays, customers look for not only tasty ice cream but the one which contains some health properties and nutritional components. Clean label products are preferred, and consumers also want to purchase environmentally sustainable and healthy ice cream. This situation has affected modern research trends. Currently, scientists try to develop new formulations of ice cream which would be functional and based on plant ingredients. In the modern world, the food industry should take into

account the ecological and nutritional concerns of consumers. Ice cream must be enjoyed and, at the same time, be environmentally friendly. This trend involves moving away from enjoying ice cream for fun. Consumers are seeking ice creams that provide more value than just indulgence. They want ice cream products that will help them live better. As a result, the ice cream industry is shifting gears to address the needs of its customers. The production of functional and plant-based ice creams is among the leading changes in this sector. These types of ice creams are gaining popularity due to their health benefits and eco-friendliness.[6] Consumers are always keen on the information they receive regarding the foods they eat. The presentation of the information is equally important. The availability of understandable information on the label is positively correlated with the purchase of the product. Research shows that there is positive correlation between the purchase of food and the presence of nutritional symbols on the front labels.[7]

The ice cream market around the world is getting bigger and bigger. It was worth seventy-five billion dollars in the year two thousand twenty three. It  is expected to keep growing at a rate of five-point two eight percent every year. This means the it will be worth one hundred nineteen billion dollars by the year two thousand thirty-two.

The part of the ice cream market that is really taking off is the ice cream segment. This includes it is made from plants has sugar and has probiotics and prebiotics. The functional ice cream segment is growing fast. It was worth two hundred forty million dollars in the year two thousand twenty-four. The functional ice cream segment will be worth four hundred forty million dollars, by the year two thousand thirty-two. It is growing at a rate of seven-point three four percent every year.[8] People are changing the way they eat. This change is happening faster. This is because of a thing: more people are paying attention to what they eat and trying to be healthy more people are finding out they are lactose intolerant more people are becoming vegan or flexitarian and more people want to eat in a way that is good, for the planet. [9]

## 1.2. Health Concerns Associated with Conventional Ice Cream

Conventional ice cream made from dairy has a nutrition profile that is being looked at closely by nutrition scientists and public health experts over the world. This type of ice cream has a lot of calories because it has a lot of sugars and saturated fats. These ingredients are linked to problems, like being overweight obesity, type 2 diabetes, heart disease and insulin resistance when eaten much. They can also lead to metabolic disorders. A lot of research has shown that eating a lot of fat is connected to a risk of many health issues. These issues include not heart and metabolic diseases but also cancer, neurological disorders, chronic respiratory problems and gastrointestinal issues.[10]

Food companies make ice cream in factories. This type of ice cream is called -processed food. The NOVA framework says that ultra-processed foods are made from ingredients that people do not usually use when they cook at home. These ingredients include things that make food taste good look nice and feel smooth. They also include things that make food taste sweet. The NOVA framework is a way to group foods, into categories. Ice cream falls into the ultra-processed foods category because it is made with these kinds of ingredients.[11] A review published in 2023 found that eating foods with UPF raised the risk of obesity by 32 percent. It also showed that eating foods increased the risk of type 2 diabetes by 37 percent, high blood pressure by 32 percent and high triglycerides by 47 percent. The study looked at UPF and its effects, on health. UPF stands for -processed food. These foods are often linked to health problems. The review was an analysis of many studies.[12] A big study that looked at 45 studies published in the BMJ in 2024 found out that people who get more Ultra Violet Radiation exposure are more likely to die from anything get heart disease, type 2 diabetes feel depressed and have bad sleep. The study really showed that more Ultra Violet Radiation exposure is not good for people. It can cause a lot of health problems, like disease and type 2 diabetes and also affect people’s sleep

and mood leading to depressive outcomes and adverse sleep-related outcomes. Ultra Violet Radiation exposure is an issue. [13] People did a study to see how eating a lot of ultra-processed food affects the risk of adult obesity. This study looked at other studies and found a clear link between eating ultra-processed food and the risk of obesity, in adults. This means that eating -processed food can really increase the risk of obesity. So it is an idea to eat food that is not processed as much. This is another reason why food companies should make food that's not as processed.[14] The quality of the food we eat is really important when it comes to how ultra-processed foods affect our health. Eating quality food can actually reduce the bad effects that ultra-processed foods have on our bodies. The quality of our diet is like a middleman, between eating a lot of -processed foods and how healthy we are. If we eat a lot of -processed foods but also eat a lot of good quality foods then the bad effects of the ultra-processed foods are not as strong.[15] These findings are really interesting. They have made companies change their products. They are taking out sugars and putting in natural ones instead. They are also taking out fats and using fats from plants. Additionally, they are adding things like fibre, prebiotics and special nutrients to make their products better, for people. [6] When we look at sweetener and seed incorporation in ice cream, we find that it works for both dairy and vegan ice cream. These substitutions can make the ice cream better in terms of quality. They can also increase the antioxidant activity. The good thing is that this does not affect how the ice cream tastes to people who eat it. Sweetener and seed incorporation in ice cream is a good way to improve ice cream. Sweetener and seed incorporation, in dairy ice cream and vegan ice cream can make these products better.[16]

Lactose intolerance is a problem, for people who love eating ice cream. It stops many from enjoying ice cream. 65% Of adults worldwide have this issue. In East Asia it affects 70 to 100% of people. In South Asia, West Africa and the Middle East the numbers range from 60 to 80%.[17] This problem comes from the fact that the body does not have enough of a helper called intestinal lactase-phlorizin hydrolase. This helper is

necessary to break down lactose; a type of sugar found in milk and other foods into things like glucose and galactose in the small intestine. When lactose is not broken down it moves to the intestine. There tiny living things like bacteria feed on the lactose and make gases, like carbon dioxide and hydrogen. These gases cause the body to react with symptoms like feeling bloated having gas getting stomach cramps and having diarrhoea. [18] Some people are lactose intolerant. Others have a milk protein allergy. This allergy is usually to casein and whey protein. It is a problem for 2 to 3 percent of people in the world. People, with this milk protein allergy need to stop eating dairy products There are also people who choose not to eat dairy products because they are vegan. Then there is the issue of the environment. Dairy farming uses a lot of land and water. It also makes greenhouse gases. So, we really need to have dairy-free alternatives. Milk protein allergies and vegan people and the environment are all reasons why we need these dairy- alternatives.[19]

## 1.3. Emergence of Vegan and Plant-Based Ice Cream

The concept of veganism, defined as the abstinence from any kind of animal products such as meat, dairy, eggs, and honey, has evolved from being a fringe dietary choice to a full-blown lifestyle change with far-reaching impacts on the entire food sector worldwide. This development stems from multiple reasons like ethics of animal cruelty, health-based motives, environmental sustainability with regard to contributions to greenhouse gas emissions, land degradation, and water usage among others.[20] Amongst plant-based meal options made from pseudocereal sources, there is nothing like quinoa. It contains all the necessary proteins in addition to amino acid content, is loaded with minerals, and is an excellent source of fiber.[21] In a recent publication in the journal Nature Food, 2023, entitled "Life cycle assessment of greenhouse gas emissions, land use, and water usage associated with dietary habits of the UK population," it conducted a life cycle assessment analysis using dietary data of 55,504 UK participants and found that vegan diets had 25.1%, 25.1%, 46.4%, and 27.0% lower greenhouse gas emissions, land

use, water usage, and eutrophication impact, respectively, than high meat eaters' diets.[22]

This supports what we had already predicted. A plant-based dietary pattern can make an enormous difference concerning greenhouse gas emissions. Notably, it may reduce them by up to 49% to 70% globally.[23] The public is very enthusiastic about researching the nutrition of what we consume and its implications for our existence. The research on plant-based diets is a growing field now. It explores the impacts of vegetarianism and veganism on our society and well-being. The practice of vegetarianism and veganism is considered legitimate and now enjoys significant attention with far-reaching implications in the academic sphere.[24] Plant-based dietary patterns have recently been identified as having multiple benefits for human health and planetary sustainability.[25]

The PBMA market has emerged as one of the most lucrative categories that the vegan trend produced, with the forecasted value of global sales expected to grow to USD 30.8 billion by 2031.[26] Vegan innovations in ice creams based on the fermentation of plant-based milk alternatives with moringa leaf flour as a novel plant-based base ingredient in frozen desserts are noteworthy examples of scientific research.[27]

Vegan ice cream enrichment with fermented hazelnut cake has been proven effective in increasing nutritional and bioactivity value of ice creams. This provides further possibilities of formulations.[28] Market research shows that, despite taste and texture playing a major role in consumer decisions, factors such as ethics, sustainability, and health concerns have become increasingly important when considering younger consumers raised in the age of environmental crisis.[29] Cross-cultural surveying has also confirmed that taste, ingredient visibility, and ethics play a major role in decision-making concerning the purchase of vegan ice creams.[30] In India, the vegan ice cream market reached a value of about thirty million dollars in 2024. In 2033, this number is projected to reach fifty million dollars. Indians have had an appreciation of plant-based and dairy foods since childhood. Vegan ice cream in India is

rapidly growing in value due to the development of new flavors. Indian ingredients including jaggery, dates, chia seeds, and millets are used in the production of these flavors. Moreover, locally-grown fruits are incorporated into the vegan ice cream. The vegan ice cream market in India holds a lot of promise.[31]

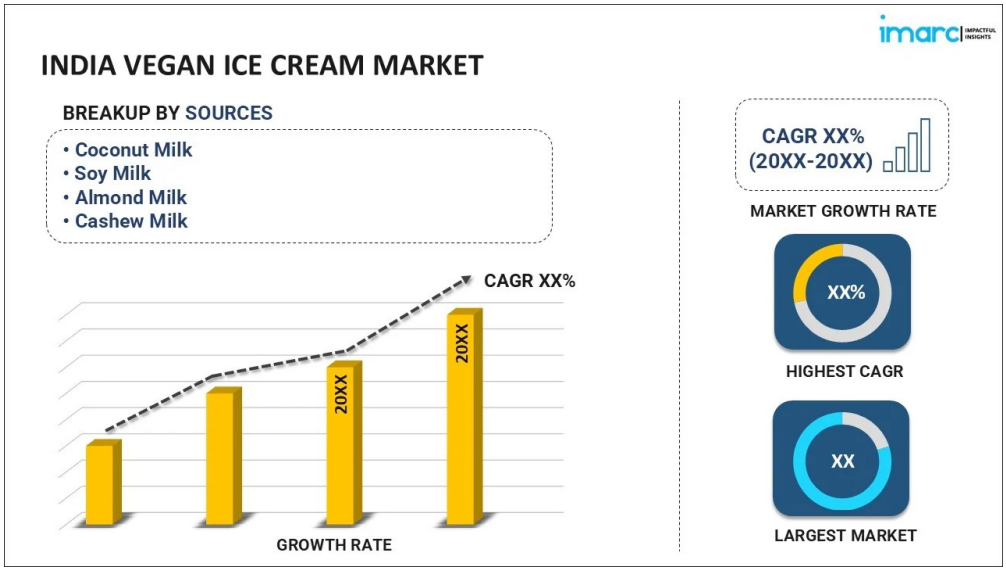



**Figure 1: Global ice cream market growth and projected CAGR**

**Market Research Future, 2024**

Vegan ice cream is a dessert made from plants. It tries to be like ice cream but uses no animal products. It is usually made with a base from plant milk. This base comes from things, like cereals nuts legumes seeds or pseudocereals. It is mixed with plant oils or fats sweeteners stabilisers and flavouring.[26] Nutritionally, well-formulated vegan ice cream is inherently cholesterol-free and may provide meaningful quantities of dietary fibre, unsaturated fatty acids, plant polyphenols, and antioxidants, which are largely absent in dairy counterparts. [18] However making plant-based ice cream is still tricky. This is because it does not have dairy proteins, like casein micelles and whey fractions. These proteins help with mixing making foam and combining fat in ice cream. Hence, the selection of hydrocolloids, emulsifiers, and plant proteins is critical for vegan ice cream makers. It is essential that those be chosen to provide a similar texture to conventional ice creams.[32] Such products are being produced using plant-based milks, such as kidney beans milk.

Furthermore, there are probiotics added to the vegan ice cream products. The ice cream based on the red kidney beans milk and probiotics shows satisfactory performance. Good characteristics.[33] Ice cream from the hemp drink based on plant proteins and guar gum has proven that selective use of hydrocolloids and proteins in this case could result in adequate physical properties.[34] It has been found that vegan ice creams formulated with probiotics and prebiotics have an acceptable count of probiotics of 6-9 log CFU/g, which complies with international standards of probiotic foods.[35] A comprehensive review on dairy-free solutions for frozen dessert applications provides necessary information on formulation techniques and other technical issues related to the discussed product line.

## 1.4 . Functional and Symbiotic Ice Cream

The idea of functional food, or food products which offer health effects above and beyond mere nutritional value, has become a cornerstone of food product innovation globally. Ice cream is an especially promising form of functional food, owing to its excellent acceptability by consumers worldwide and unique physicochemical properties — the emulsified oil-in-water suspension with a high proportion of aqueous phase content — which allows the encapsulation and stabilization of various bioactives throughout processing and freezing stages. A systematic review of the use of functional ingredients in ice cream has shown that their addition reliably resulted in increased nutritional value, altered sensory characteristics, and positive physiological results when probiotics and prebiotics were added.[37] Ingredients used in functional ice cream include dietary fibre, inulin, FOS, plant polyphenols, bioactive peptides, phytosterols, carotenoids, vitamins, as well as whole functional components like algae, seeds, and new plant flours.[6]

The ISAPP says that Prebiotics are things that the tiny living things inside our body use to help our body be healthy. Prebiotics are used by these living things to make our body healthier. The ISAPP is talking about Prebiotics. How they work with the tiny living things, in our body

to give us health benefits.[38] A food ingredient is a prebiotic if it does three things. First the prebiotic has to be able to withstand the acid in the stomach and the enzymes that break down food in the body. The prebiotic also has to be able to be broken down by the bacteria in the colon. The prebiotic has to help the good bacteria in the body grow and be more active which makes the body healthier. The prebiotic does this by helping the bacteria that are already, in the body like the beneficial microorganisms to work better and make the body stronger. This is what makes the prebiotic a prebiotic. [39] A review of inulin-type fructans published in Advances in Nutrition in 2022 summed up evidence from trials. It showed that inulin-type fructans have prebiotic effects on gut bacteria. They help increase the growth of bacteria like Bifidobacterium, Lactobacillus and Faecalibacterium prausnitzii. This leads to health benefits. These benefits include an intestinal barrier, better bowel movements and improved insulin sensitivity. Inulin-type fructans also help triglycerides and improve cholesterol levels. They increase the absorption of calcium and magnesium in the body. They also ensure satiety for an extended period of time. This study proves the usefulness of inulin-type fructans in terms of health.[40] Intervention studies on humans have proven that the administration of inulin-type fructans leads to beneficial changes in the composition of the gut microbiome, particularly increasing the number of bifidobacteria.[41]

The composition of harmful bacteria in our stomach is deteriorating due to our dietary habits. We consume too much junk food and insufficient fiber content. This has led to an increase in gut problems worldwide. These problems are connected to long-term diseases like type 2 diabetes, heart disease being bowel disease. A review in the Journal of Clinical Medicine in 2025 looked at studies that showed prebiotics, probiotics and a mix of both, called symbiotic can help in ways. They help good bacteria grow make short-chain fatty acids that feed the cells in our colon and help our system. They also make our gut stronger. Reduce inflammation by affecting a specific pathway, in our body.[42] Incorporating prebiotic fibres into widely consumed food products such as ice cream therefore

constitutes a strategically viable and evidence-based public health approach. [43] Inulin has similarly been demonstrated to support the growth of vegan yoghurt cultures in pea protein-based frozen dessert systems, simultaneously enhancing textural properties and prebiotic activity.[44]

The role of prebiotics in ice cream is really important. Prebiotics like inulin are very useful. Inulin makes the ice cream mix thicker stops ice crystals from growing when you store the ice cream and helps it melt slowly. It also makes the ice cream lighter and fluffier because it can form a gel. This means you can use fat in the ice cream and it will still taste good. For example, some researchers found out that prebiotic fibers, inulin and apple fiber help the good bacteria in the ice cream stay alive when it goes through your body. They did an experiment in a lab to see what happens to the bacteria when you eat the ice cream. The researchers discovered that by combining prebiotics and probiotics, the beneficial microorganisms could flourish. Additionally, other ingredients like xylitol and guar gum are incredibly helpful in plant-based ice creams. The former ensures that the consistency of the ice cream stays smooth and prevents crystal formation, whereas the latter acts as a bacterial substrate, both in vivo. This is beneficial since it can help maintain one's well-being.[43] Psyllium mucilage has also been tested as an alternative stabilizer for vegan yogurt ice cream, resulting in significant advancements in the physical firmness and sensory texture of the product.[45]

## 1.5 . Plant based milk substitutes (PPBS)

Plant-based milk alternatives are plant-derived aqueous preparations that have been refined, homogenized, and stabilized to mimic the properties of milk from animals in terms of its appearance, consistency, and nutritional value where feasible. In technical terms, they are colloids in which an aqueous phase is the dispersion medium for starch, proteins, lipids, and other cellular debris derived from the plant material. Beyond the obvious rationale of vegan concerns, there is a strong structural need

for plant-based milk alternatives as evidenced by the lactose intolerance of 65% of the adult population worldwide, increasing incidence of milk protein allergies, and proven environmental impact associated with the manufacture of animal milk, in the form of greenhouse gas emissions, land use, and freshwater use.[20] Numerous life cycle assessment studies confirm the fact that all types of plant-based milk alternatives contribute significantly fewer greenhouse gas emissions, require far less agricultural land and freshwater, and cause less aquatic eutrophication than their animal-derived counterparts.[46]

Plant Based Milk Substitutes are made from lots of kinds of plants. We get them from cereals like rice, oat, spelt, kamut, corn and rye. We also get them from nuts like almond, coconut, walnut, hazelnut, pistachio and tiger nut. Then there are pseudocereals like quinoa and amaranth. Plant Based Milk Substitutes also come from legumes, which include soy, pea, cowpea, peanut and kidney bean. We even get them from seeds, like flax, sesame, hemp, sunflower and pumpkin.[20] The nutritional characteristics of Plant Based Milk Substitutes vary a lot depending on the plant they come from and how they are made. Plant Based Milk Substitutes like the ones made from soy are pretty close to cow milk when it comes to the amount of protein and the quality of acids they have. On the hand Plant Based Milk Substitutes made from almonds have very little protein in them. The ones made from oats are special because they have something called beta-glucan which helps lower the cholesterol in our blood. These are made from hemp are sources of essential fatty acids and they have just the right amount of omega-6 and omega-3. One big problem with Plant Based Milk Substitutes compared to cow milk is that they do not have as much of some important nutrients like calcium, vitamin D, vitamin B12, riboflavin and an amino acid called lysine that we find in the ones made from grains. We can fix these problems by adding these nutrients to the Plant Based Milk Substitutes on purpose and, by mixing plant sources together to get a more complete mix of amino acids.[47]

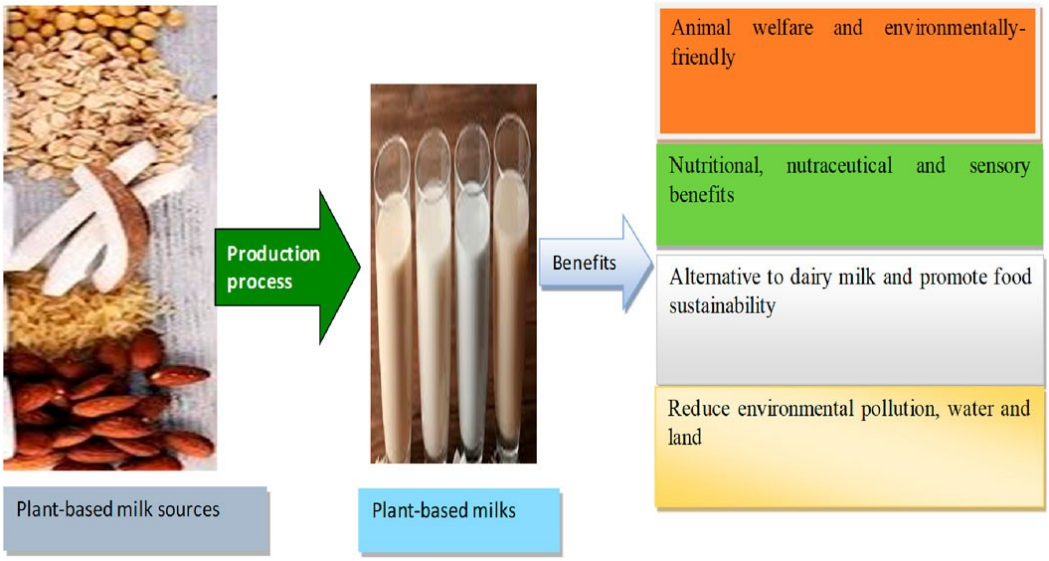



**Figure 2: Sources of plant-based milk substitutes (PBMS).**

**Source: Irondi EA et al., Frontiers in Food Science and Technology, 2025**

When you look at Peanut Butter Made Snacks or PBMS you see that they have a lot of things in them. These things include acids and flavonoids and phytosterols and bioactive peptides and dietary fiber. All of these attributes in PBMS are known to contribute to the antioxidant, antihypertensive, anti-inflammatory, and antidiabetic effects observed experimentally. These are some of the findings that experimental research on PBMS has revealed.[20] The development of advanced technologies such as high-pressure homogenization, ultrasonication, enzymatic hydrolysis, and fermentation has proven highly effective in enhancing the stability, nutrient bioavailability, and sensory acceptance of PBMS, thereby increasing the number of plant sources available for high-quality commercial production.[38] With the predicted rise in global population to 10 billion people by 2050, and its increasing tendency towards urbanization, there will be a need for food production systems that are nutritious and sustainable. PBMS and their functional foods, including plant-based ice cream, represent one potential solution to meeting these requirements.[20]

## 1.6 NUTRITIONAL AND FUNCTIONAL POTENTIAL OF SELECTED PLANT BASED INGREDIENTS

### 1.6.1. AMARANTH

Amaranth (Amaranthus spp.) is a genus of approximately 60 species within the family Amaranthaceae, classified as a pseudocereal on the basis of its seed morphology, culinary usage, and nutritional profile despite its non-membership of the grass family Poaceae. [48] The three species historically cultivated for grain production are Amaranthus cruentus (red amaranth), Amaranthus caudatus , and Amaranthus hypochondriacus , all of which were central crops for pre-Columbian civilizations in Mesoamerica before European colonization.[48] Today people are really interested in amaranth again. The FAO of the United Nations also known as the FAO thinks that amaranth is one of the promising crops to help with food problems around the world. This is because amaranth is very good, for you it does not have. It can grow well in tough conditions. Amaranth can handle not having water it can handle salty soil and it can even handle very high temperatures. These things are very important now because of climate change. Because we do not have as much water as we need.[49]

The amount of protein in amaranth grain is around 15.4 to 16 percent when it is dry. This is really good compared to grains like wheat which has 13.5 to 14.5 percent protein, maize with 10.6 to 13.8 percent, barley with 10 to 14.9 percent and oat with 12.4 to 12.9 percent. Amaranth protein is very special. It has a lot of lysine which's an important part of protein. Amaranth has 55 to 65 milligrams of lysine per gram of protein. This is two times more than wheat and three times more than maize. This makes amaranth a great addition to diets that are based on plants because lysine is often the part that's missing. Amaranth has a lot of

amino acids around 47.6 grams per 100 grams of protein. This is a lot more than what the FAO and the WHO say we need to be healthy. Amaranth also has a lot of cysteine and methionine which're important for our bodies. These are often hard to find in proteins from beans. This means amaranth is a match, for both grains and beans when we mix them together.[48]

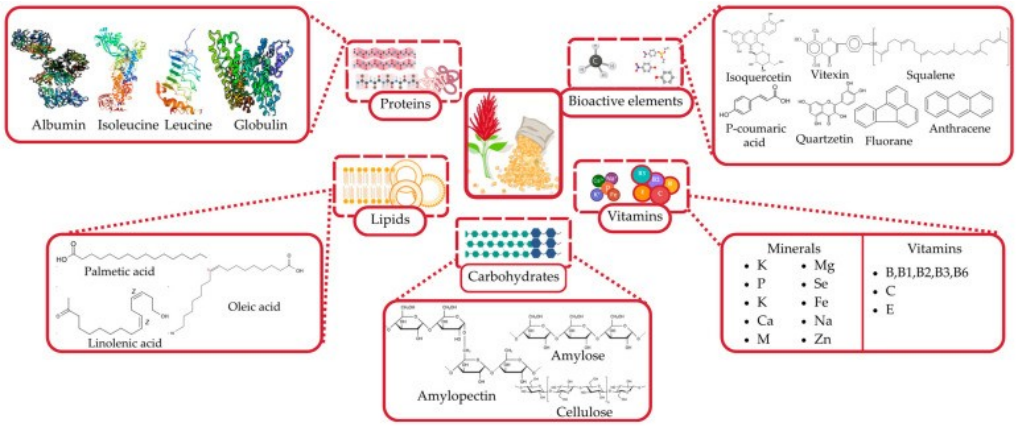



**Figure 3: Nutritional and phytochemical composition of amaranth grain.**

**Source: Toimbayeva D et al., Foods, 2025.**

Amaranth grain has a lot of protein in the perisperm areas. This is different from grains, where the protein is mostly in the endosperm. The way protein is spread out in amaranth grain makes it very good for you. It has a high protein efficiency ratio, which's around 1.5 to 2.0 and a biological value of about 75. This is as good as or even better than other grains.

The fat in amaranth grain is also very good for you. It makes up about 6 to 9 percent of the grains weight. Some types of amaranth grain can have much as 19 percent fat. The good thing about this fat is that it has a lot of fatty acids, which are good for your health. Most of the fatty acids in amaranth grain are acid, which is about 50 to 62 percent. Then there is acid, which is about 16 to 26 percent.

Amaranth grain also has something called squalene, which's very good for you. It helps keep your liver healthy boosts your system and protects

your skin. Squalene is also an antioxidant, which means it helps keep you from getting sick. It is very rare to find a food that has much squalene. Amaranth grain also has something called tocopherols which're like vitamins that help keep the fat in the grain from going bad.

Amaranth grain has a lot of good things in it too. It has flavonoids, like quercetin and kaempferol which're like special helpers that keep you healthy. It also has acids, like ferulic acid and gallic acid, which are good for you. Then there are betaxanthins and betacyanins like amaranthine, which give the grain its color. Amaranth grain also has carotenoids, like lutein and zeaxanthin which're good for your eyes.

All these special helpers in amaranth grain can do a lot of things for you. They can help lower your blood pressure stop tumors from growing and keep your blood sugar under control. They can also help boost your system and keep you from getting sick. Some studies have even shown that peptides, from grain can help keep you from getting hypertension, diabetes and dyslipidemia.[49]

**Table 1 : Proximate Composition and Mineral Content of Amaranth Grain (per 100 g dry weight) [18,21]**

| Proximate Component | Range / Value | Mineral / Antinutrient | Range / Value |
|---|---|---|---|
| Protein (%) | 15.4 – 16.0 | Magnesium (mg/kg) | 2300 – 3360 |
| Fat (%) | 5.6 – 9.7 | Calcium (mg/kg) | 1300 – 2850 |
| Ash (%) | 2.5 – 3.2 | Sodium (mg/kg) | 160 – 480 |
| Dietary Fibre (%) | 3.1 – 6.6 | Iron (mg/kg) | 72 – 174 |
| Carbohydrates (%) | 60.0 – 69.0 | Zinc (mg/kg) | 36.2 – 40.0 |
| Energy (kcal/100 g) | 370 – 380 | Phytic acid (g/kg) | 2.9 – 7.9 |
| Squalene (% of oil) | 6.0 – 8.0 | Saponins (mg/kg) | 0.9 – 4.91 |
| Total Essential AA (g/100g | 47.6 | Trypsin inhibitors | Trace amounts |

| Proximate Component | Range / Value | Mineral / Antinutrient | Range / Value |
|---|---|---|---|
| protein) | | | |

Although amaranth's nutritional profile is outstanding, the grain does contain antinutritional factors — tannins, phytates (2.9–7.9 g/kg), saponins (0.9–4.91 mg/kg), oxalates, and nitrates (concentrated in leaves, not grain) — that can reduce micronutrient bioavailability and impart undesirable bitter or astringent sensory notes.[48] However, these antinutrients are highly responsive to standard food processing interventions, particularly germination. Research has demonstrated that 48-hour germination at 26°C increases protein availability by 8%, total energy by 11%, and linoleic acid content by 10%, while reducing resistant starch by approximately 70%, indicating substantially improved starch digestibility and protein bioavailability. [50]

Combined soaking and germination treatments have been shown to produce significant reductions of 32.30% and 29.57% in tannin and phytic acid contents respectively, and to simultaneously increase crude protein, crude fibre, total phenolic content, and antioxidant activity. [51] Fermentation with lactic acid bacteria provides complementary benefits: it has been shown to reduce phytic acid from 11.06 to 1.00 mg/g and tannins from 4.92 to 2.05 mg/g, while increasing fat, protein, and ash content by 3%, 22%, and 14%, respectively. [48] Innovative non-thermal processing technologies including cold plasma, high hydrostatic pressure, pulsed electric fields, and ultrasonic processing have also emerged as efficient strategies for antinutrient reduction in amaranth. [49]

Amaranth has proven to be very successful in the manufacture of an impressive array of different foods. Partial replacement of wheat flour with amaranth flour at 15-30% enhances the protein, mineral composition, and nutritional value of the resulting bread. Sensory analysis results show acceptable consumer acceptance. Gluten-free amaranth flour when substituted at 25% in cookies enhances the

essential amino acid composition, mineral content, and physical properties of the cookie. Sports beverages made from amaranth provide better cycling performance than commercially available sports drinks due to better amino acid and mineral composition.[48] Amaranth has also been used in pasta, noodles, extruded snack products, baby food products, gluten-free nutritional supplements, and functional beverages with positive results in terms of nutritional improvement.[49] The extensive review on nutraceuticals and bioactive substances present in quinoa and amaranth has provided additional evidence on their common wealth in phenolic acids, flavonoids, and antioxidant peptides, thus corroborating the functional food properties of both pseudocereals.[52] Modern studies have revealed promising trends in terms of encapsulation of amaranth proteins for efficient delivery of bioactive substances, and development of amaranth edible films and coatings as an ecological solution in food packaging. As for the current investigation, amaranth milk obtained by soaking, germination, grinding, filtration, and pasteurization of amaranth grains represents the major plant milk base due to its high digestibility, low content of antinutritional factors, increased bioavailability of nutrients, and high bioactive substance content.[49]

**Table 2: Key Bioactive Compounds in Amaranth Grain and Their Documented Health Activities [18,21]**

| Compound Class | Specific Compounds | Documented Health Activities |
|---|---|---|
| Phenolic acids | Vanillic, gallic, ferulic, caffeic, syringic acid | Anti-cancer, anti-obesity, anti-diabetic, anti-inflammatory, antioxidant, anti-bacterial |
| Flavonoids | Quercetin, kaempferol, rutin, vitexin, isovitexin | Antioxidant, antifungal, anti-carcinogenic, anti-inflammatory, hepatoprotective, neuroprotective, cardioprotective |

| Compound Class | Specific Compounds | Documented Health Activities |
|---|---|---|
| Betacyanins | Amaranthine | Antioxidant, antimicrobial, cytotoxic |
| Carotenoids | Lutein, zeaxanthin, β-carotene | Antioxidant, anti-diabetic, anti-inflammatory, anti-cancer, anti-osteoporosis, vision-protective |
| Tocopherols | α, β, γ, δ-tocopherols | Antioxidant, cardioprotective, anti-cancer, anti-diabetic, anti-obesity |
| Squalene | Triterpenoid squalene | Antioxidant, hepatoprotective, immunostimulatory, skin-protective |

### 1.6.2. MAKHANA (FOXNUTS )

Makhana (Euryale ferox Salisb.), vernacularly known as foxnut or gorgon nut, is an aquatic plant of the family Nymphaeaceae that produces nutritionally dense seeds within submerged fruits. It grows in slow-moving freshwater bodies—ponds, lakes, and flooded depressions—primarily across the Indian subcontinent and Eastern China, with Bihar state in India accounting for approximately 80–90% of national production. Makhana has been. Eaten in India and China for more than three thousand years. It is very important in medicine, traditional Chinese medicine and religious rituals. In Ayurveda Makhana is thought of as a tasting, heavy and strength building substance. It is given to people who're weak have diarrhoea, urinary problems and reproductive health issues.

In Chinese medicine Makhana is valued because it is good for the spleen and kidneys and has astringent properties. To make Makhana seeds into a snack they are heated to a temperature until they pop. This makes the seeds big and white. People like to eat them. Makhana seeds are also

used in traditional dishes like kheer and raita. If we look at the nutrients in 100 grams of popped Makhana seeds we can see that they have 11.16% protein, 75.04% carbohydrates and a little bit of fat. Makhana seeds also have some calcium, phosphorus, magnesium, iron, zinc and potassium which're all good for us. Makhana is a food to eat because it has a lot of nutrients and it is good, for our health.[53] The protein quality of makhana is exceptional: it shows an  (EAAI) of 89–93%, which surpasses many common cereal grains, and possesses an arginine + lysine to proline ratio of 4.74–7.6, a parameter associated with cardiovascular health support. [54] Its mineral profile is characterized by low sodium and high potassium content, contributing to blood pressure regulation support, and very low monounsaturated fat content, making it appropriate for blood sugar management. [53]

The antioxidant content of makhana varies notably with processing: raw seeds have higher phenolic content and DPPH radical scavenging activity compared to popped seeds, as high-temperature processing degrades heat-sensitive phenolic compounds. The principal free phenolics in raw makhana seeds include catechin (1.806 mg/100 g) and gallic acid (1.336 mg/100 g). [55]

Makhana is really good for you. It is not just because of what it contains. The things that makhana can do to help your body are very interesting. It helps keep your blood sugar levels in check which's great for people with diabetes. Makhana also helps you feel full so you do not eat much. This is because makhana has a lot of something called starch and it does not raise your blood sugar levels too quickly. Makhana is also very high in fiber, which's good for your intestines and helps you go to the bathroom regularly. Makhana has things in it called flavonoids and phenolic compounds like catechin and gallic acid. These things help keep your body safe, from damage. May even help keep your heart healthy and prevent cancer. It is one of the health foods people should consider eating.[56] Aquatic edible plants' polyphenol content and their antioxidant activities have been investigated and classified as sustainable sources of

bioactive compounds, thus classifying makhana among other edible aquatic plant components that offer nutritional benefits.[57] In ancient reproductive medicine, it is used in increasing the number and quality of sperms, while Chinese medical texts prescribe formulations of makhana to treat spermatorrhoea. It is believed that the reproductive benefits of this plant result from its moisture balancing and hormone-related properties.[58] The fact that makhana contains no food allergens, especially the top eight allergenic foods, makes it ideal for producing allergen-free food items.[59] It has also been considered in formulating foods, where it has proven to be acceptable in terms of taste, among other aspects.[60] Its functional properties as an ingredient in value-added foods have also been systematically reviewed, indicating improvements in protein utilization, antioxidant property, and textural attributes.[61]

### 1.6.3 JACKFRUIT SEED POWDER

The fruit that the trees bear all around the world is called jackfruit belonging to the Moraceae family. The jackfruit tree thrives in slightly cool climatic regions in south, southeast Asia, east Africa and parts of Latin America.

India emerges as the leader in producing the maximum amount of jackfruit annually accounting for about 1.8 million tonnes per year. Some of the Indian states that have jackfruits growing are Assam, West Bengal, Uttar Pradesh, Maharashtra, Kerala, Tamil Nadu and Karnataka.[62] Jackfruit's fruit anatomy consists of a hard outer spiny rind, stringy inner rags, yellow-colored pulpy arils which are the edible portions and several large seeds surrounded by whitish fibrous coating. While the arils receive a high demand among consumers, the seeds, accounting for around 10 to 15 percent of the fruit's weight, end up getting discarded as agricultural waste, and a shocking 90 percent of all the seeds produced around the world end up getting wasted.[63] This wasted seed material is a tremendous loss of potential nutrition and a missed chance of value addition for the circular agricultural economy.[64] Favorable physicochemical and sensory properties were observed after evaluating

quality attributes of value-added foodstuffs made using jackfruit seed flour.[65]

Nutrition-wise, jackfruit seeds are very rich. Jackfruit seeds have about 7 grams of protein in 100 grams of its quantity. There are 1 gram of fats. Also, there is 1.5 grams of fibre. Grams of carbohydrates in the seed of Jackfruit are 38. Calories in them are 184. Jackfruit seeds have many mineral elements. About 246 milligrams of phosphorus. Magnesium is 200 milligrams. Calcium is 33 milligrams, zinc and iron is one milligram. These seeds contain vitamins. There are vitamin B1 Thiamine and vitamin B2 Riboflavin. These nutrients fight disease. I have described here the jackfruit seed and their nutrients. These nutrients are beneficial to our health. These minerals and vitamins in the seeds of jackfruits are very necessary.[62] According to a 2025 review, jackfruit seeds were found to have starches of 12.86–17.90 g/100 g and proteins ranging from 18%, dietary fiber was found to be of 1.56–3.92 g/100 g.[66]

Major protein Jacalin, which is a tetrameric lectin that only exists in the seeds of jackfruits, has been extensively studied regarding its ability to stimulate T lymphocytes and modulate immune responses in immunology. This protein is used in HIV immunity evaluation and cancer immunology.[62] The seeds of the jackfruit have a lot of things in them that can affect our bodies. These things are called compounds and they include catechin, epicatechin, ferulic acid, rutin, gallic acid and caffeic acid. All of these compounds together are what make the jackfruit seeds have an antioxidant capacity. This is what the tests, like DPPH, ABTS and FRAP assays have shown. [67] Anticancer, antiulcer, antihypertensive, and anti-aging effects have been associated with lignans, isoflavones, and saponins contained within the seeds of the fruit.[64] There are some positive features of the jackfruit seeds to be considered. The first feature of the seed of this fruit is that it can reduce inflammation. Another feature is that jackfruit seed has antibacterial properties. Moreover, it provides something useful for microorganisms which are needed by our body. It serves as their food, namely inside our stomach. That is why our

body will receive what it needs for its own needs.[68] High content of magnesium in the fruit seeds will help to regulate blood pressure.[67]

In relation to the applications of functional foods, both the jackfruit seed flour and powder have shown promising technological attributes such as high water absorption capacity (WAC), oil-holding capacity (OHC), swelling index, and emulsification capacity.[64] In 2023, it was shown that jackfruit seed powder can be effectively used to improve the nutritional value of yoghurt products by adding protein, minerals, and antioxidants.[69] Processing of jackfruit seeds by autoclaving, boiling, or controlled drying at 50–60°C followed by grinding effectively reduces trypsin inhibitors and tannins while preserving protein quality. [37] The low cost, local availability, and inherent sustainability value of utilising otherwise-wasted jackfruit seeds in value-added functional food products — reducing agricultural waste while enhancing nutritional density — make jackfruit seed powder an ideal ingredient for the prebiotic plant-based ice cream formulation proposed in the present study. [63] The feasibility of jackfruit seed milk as a base for vegan ice cream has been demonstrated in prior studies, establishing a foundational precedent for its use in plant-based frozen dessert innovation.[70]

### 1.6.4. GOND KATIRA (TRAGACANTH GUM )

Gond Katira, known internationally as tragacanth gum, is the dried exudate obtained from incisions made in the stems and branches of Astragalus gummifer Labillardière and related Astragalus species of the family Fabaceae, native to the mountainous arid regions of the Middle East and Central Asia, including Iran, Turkey, Syria, and Afghanistan. [71] It is one of the oldest food-grade hydrocolloids used since ancient times for its use in various food, medical, and cosmetic applications that have been used for millennia. The dried exudates appear as light-colored and translucent ribbons or flaky masses that do not exhibit any odors and flavors when dry; once hydrated, they absorb several times their weight in water to form gels and mucilages that contribute to the functionality of these products.[72] Many families and cultures residing in Middle Eastern

countries believe that Gond Katira is essential in summers. They prepare drinks by soaking the product in milk, water, or even sweet sherbets, which help them cool their body, facilitate proper digestion, and support various other processes. In the field of medicine, Gond Katira is termed 'Sheetala', which translates to cooling. It is used for balancing 'Pitta dosha', especially for patients suffering from burning sensations, excessive thirst, and urinary problems.

Gond Katira gum is made up of two constituents – tragacanthin and bassorin. Tragacanthin dissolves in water and assists in maintaining stability and uniformity of the mixture. Bassorin acts as a gel-making constituent which contributes to viscosity formation. The gum is made up of several sugar molecules such as arabinose, galactose, xylose, fucose, and galacturonic acid.[72] As a nutrient component, for every 100 g of Gond Katira gum, one gets an estimated amount of 70 kcal, 35 g carbohydrates, and 30 g dietary fiber.[71] As far as stability is concerned, the gum shows great stability in the pH range of 2–8 and a certain temperature range and hence can act as a viscosity enhancer as well as a gelling agent in both acidic and basic foods; this property makes it more valuable in the manufacture of ice cream.[73]

Gond Katira gum is compatible with frozen dessert preparations.When you add it to the ice cream mix it makes the mixture thicker. This helps to slow down the formation of ice crystals as the mixture freezes gets harder and is stored. As a result you get an ice cream with a more even texture. The ice cream also feels creamy in your mouth. Doesn't melt as quickly. This is similar to fat dairy ice cream but without using artificial additives. This approach fits with the trend of using labels in product development. Gond Katiras properties make it ideal for use, in desserts. It helps to create a high-quality ice cream that consumers will enjoy. The use of Gond Katira supports the creation of clean-label products.[72]

Its pharmacological applications in traditional systems of medicine include treatment of pharyngitis, diarrhea, gonorrhea, dysentery, and trachoma.[73] Gond Katira is resistant to hydrolysis by gastric acid—a

property conferred by the bassorin fraction—and is subsequently degraded by anaerobic microflora in the colon, establishing the mechanistic basis for its prebiotic potential and its suitability as a carrier for colon-targeted drug delivery systems.[73]

Recent studies on chemically-modified polysaccharides from gum katira have widened the application field of this natural gum by exploiting its potential in drug delivery systems, wound healing formulations, and edible coatings, among others, proving that this age-old natural material still has commercial significance in modern times.[73]

### 1.6.5. MONKFRUIT SWEETENER

It is crucial to decrease the intake of refined sugars due to the connection between excessive refined sugars and diseases such as obesity, diabetes, and dental cavities. Thus, many industries and individuals are moving toward zero-calorie sweeteners.

Currently, researchers pay close attention to artificial sweeteners. It is essential to find out whether such artificial sweeteners are safe for consumption and examine their potential impacts on the human body. For example, article in Nature showed that certain artificial sweeteners, such as aspartame, sucralose, and saccharine, could affect the microbial environment in the human intestines and increase glucose intolerance. Subsequent investigations on people brought more questions regarding the possible impact of such substances on human metabolism.[74] Some artificial sweeteners were shown to be paradoxically associated with higher body mass index, insulin resistance, and even type 2 diabetes in epidemiological studies — which was ironic considering the intention of using such compounds as aids in managing diet-related conditions.[75] These findings created a strong demand for natural sweeteners that would not be dangerous, have no calories, be compatible with gut microflora, and lack the safety risks typical of artificial sweeteners.[76]

Monk fruit, also known as Siraitia grosvenorii Swingle is a round greenish-brown fruit. It comes from the Guangxi Zhuang Autonomous

Region in China. This fruit is considered one of the natural sweeteners that are very sweet. In Chinese it is called 'Luo Han Guo'. The name comes from monks who grew it many years ago. The fruit belongs to the gourd family. People, in China have used fruit in traditional medicine for a long time. They use it for problems and digestive issues. Some people also think it helps them live longer. The fruit has good things in it. These include triterpenoids, flavonoids and vitamins. It even has proteins and oils that can evaporate. Because of these monk fruit is considered a food with many benefits. These benefits have been documented in studies.[75]

The monk fruit sweetness comes from compounds called mogrosides.These are made up of a base called mogrol and different amounts of glucose units attached. Mogroside V is the sweet part. Mogrosides are 100 to 250 times sweeter than sugar. Some are 250 to 400 times sweeter. The mogrosides are separated from the sugars in the fruit during processing. This results in a sweetener with zero calories from carbohydrates.  It also has an index of zero. This makes monk fruit extract great for people with diabetes or insulin resistance. The US Food and Drug Administration said it is safe to use in 2010. It is okay to use in food and drink products. When using fruit sweetener it works best when it is not more than 25% of the product. If it is more, than that it can taste bitter and metallic. This is because of the mogrol base. It can make the product less enjoyable to eat or drink.[75]

Research further demonstrated that natural sweeteners, including monk fruit, support the viability and metabolic activity of probiotic lactic acid bacteria, making them particularly suitable for use in symbiotic functional food formulations such as the prebiotic vegan ice cream proposed in the present study. [76]

The health benefits of fruit are really great and it is not just useful as a sweetener that has no calories. Monk fruit has properties that can help with a lot of things like stopping coughs helping people with asthma. It has antioxidants that can protect the liver and help keep the immune system working well. It can also help lower blood sugar. Might even help

prevent cancer. A study done by Ban and others in the year 2020 looked at what happens when you put fruit extract in yogurt. They found out that it makes the yogurt have antioxidants, which is a good thing. The yogurt also had a texture and consistency and it was still good after it was fermented. This means that monk fruit can be used in a lot of products like dairy and non-dairy foods and it will still be good, for you.[77] People want to be healthy. They like natural sweeteners such as monk fruit. This is because monk fruit is good, for people who want to eat sugar take care of their blood sugar levels keep their gut healthy and only eat foods with simple ingredients. Monk fruit is a sweetener that meets all these needs.[78]

**Significance of the Study**

1. This research develops a functional vegan ice cream by incorporating germinated amaranth milk, providing a high-quality and easily digestible protein source that addresss the low protein content in most existing vegan ice creams.
2. Foxnut and jackfruit seed starches are added as natural prebiotic sources to ensure digestive well-being, and tragacanth gum increases product stability and fiber content for metabolic well-being.
3. The formula caters to the nutritional requirements of different demographics, including vegans, lactose intolerants, high-protein athletes, and older people who need more proteins.
4. The incorporation of nuts and dates increases the nutritional value of the ice cream, contributing to fat content, mineral content, antioxidant content, and low glycemic natural sugar content.
5. Using lesser-known crop products such as foxnut and jackfruit seed powder is an environmentally sustainable approach that can minimize food waste, as well as using underutilized agricultural produce in innovative ways within the growing plant-based industry.

# AIM AND OBJECTIVES

## AIM:

To formulate a prebiotic plant-based protein rich ice cream using amaranth milk, foxnut, jackfruit seed powder, tragacanth gum.

## OBJECTIVES OF THE STUDY:

## PRIMARY OBJECTIVE

- To standardize and develop a prebiotic vegan high-protein ice cream using germinated amaranth milk, foxnut, and tragacanth gum.

## SECONDARY OBJECTIVE

- To conduct market survey for existing plant-based, prebiotic and high protein ice creams
- To prepare and standardize a prebiotic, plant-based high-protein ice cream.

- To analyze the physicochemical properties, proximate composition and antioxidant potential of the developed product.
- To conduct the sensory evaluation of the developed ice cream for taste, texture, and overall acceptability.
- To assess the cost and shelf life of developed product.
- To design packaging and a clear nutrition label that highlights the product’s benefits.

# CHAPTER - II

# REVIEW OF LITERATURE

# REVIEW OF LITERATURE

**Devalekar and Udachan (2025)** [79] aimed to create a healthy vegan ice cream. Coconut milk, date syrup, and fructooligosaccharide (FOS) were used to create it. Individuals seek out plant-based and low-sugar sweets, prompting the creation of this ice cream. Coconut milk was selected due to its fat content. Date syrup served as a sweetener that is low in sugar content. FOS was included as a fibre that promotes the growth of beneficial bacteria in your intestines. It also substitutes certain sugar. They employed a design to evaluate varying quantities of coconut milk, date syrup, and FOS. They examined how these components influenced the texture, flavour, and colour of the ice creams. They also examined aspects such as its melting properties and antioxidant effectiveness. The optimal combination was determined to be 50% coconut milk, 11.32% date syrup, and 5.81% FOS. This particular ice cream had flavor and texture. Some of the specific details include: overrun which was 65.14%. Melting rate was 40.85%. Moisture percentage was 66.32%, fat was 18.5%, and protein content was 2.93%. The ice cream was composed of various elements, for example, total phenols were 79.85 mg GAE/100 ml, while antioxidant activity was 54.80%. Individuals enjoyed the flavor, consistency, and creaminess of this plant-based ice cream. In certain aspects, it surpassed dairy ice cream. This research indicates that it's possible to create a vegan prebiotic ice cream utilizing coconut milk, date syrup, and FOS. These components offer health advantages. Ensure the ice cream is flavourful. The research demonstrates that FOS acts as a prebiotic in this ice cream.

**Aydemir *et al.* (2025)** [80] explored the valorization of fermented hazelnut cake — a protein-dense byproduct from hazelnut oil extraction processed through solid-state fermentation using Aspergillus oryzae — as a functional enrichment component in vegan ice cream, aiming to create a nutritionally improved, sustainable plant-based frozen treat. Three

recipes of ice cream – dairy ice cream (DIC), vegan with unfermented hazelnut cake (HIC), and vegan with fermented hazelnut cake (FHIC) – were prepared and fully studied for their proximate composition, texture, rheology, melting, antioxidant activity (DPPH test), volatile compound composition, ACE inhibition activity, and sensory properties both before and after the simulation of in vitro digestion process. Fermentation considerably increased the amount of proteins (FHIC: 64.64% vs. HIC: 58.02%) and resulted in unique volatile compounds formation (benzaldehyde and 3-methyl-1-butanol); all samples showed the non-Newtonian shear-thinning behavior. Overrun was found to be higher for DIC (15.39%) as compared to the vegan ice creams (4.01-7.00%). In addition, FHIC exhibited considerably higher antioxidant activity (4.73 µmol TE/g) and ACE inhibitory potential (4.85-7.42%) than DIC did. Finally, the amount of energy contained in the vegan ice creams was considerably less (18-30%) than DIC. Overall, the results of the study show that fermentation of plant by-products leads to creation of highly nutritious vegan ice creams. This is because the results from the study relate to the current investigation since they show that bases containing plant proteins have nutrient values equivalent to or even higher than those found in conventional ice creams made using milk, something that was expected from the amaranth milk used here.

**Fransisca *et al.* (2025)** [81] tried to formulate a nutritionally fortified vegan ice cream using the tempeh milk as the principal component, alongside the moringa leaf flour, which is rich in terms of vitamin, mineral, and amino acid contents. The production of tempeh milk involved soaking, milling, and straining of the product, which, in turn, was used in making ice creams of different flavors, incorporating various concentrations of the moringa leaf flour. These products were analyzed in terms of proximate composition (moisture, protein, lipid, carbohydrate, ash) as well as antioxidant activity (using the DPPH assay) and hedonic scale-based sensory acceptance. Notably, increasing levels of moringa

leaf flour significantly increased protein, iron, calcium, and total phenolic contents as well as the ability of DPPH radicals scavenging; however, higher amounts of moringa flour reduced the sensory scores due to the color and bitter taste, but acceptability was acceptable at lower concentrations, while the use of the tempeh milk resulted in an improved protein digestion rate and a unique umami taste. The study proved that milk from plants which is high in protein content, along with the advantageous green element, can produce a better nutritionally improved vegan ice cream, which is applicable to this research since amaranth milk, which is germinated, forms the base of this vegan ice cream.

**Wyrwisz and Pokorski (2025)[82]** investigated the potential of psyllium mucilage (PM), an eco-friendly hydrocolloid rich in arabinoxylan, obtained from Plantago ovata seed coats, as a plant-derived stabilizer in vegan yogurt ice cream, studying its influence across three concentrations (0.25%, 0.5%, and 0.75%), compared with the standard stabilizers guar gum (GG) and carob gum (CAR), on rheology, overrun, melt-resistance, color, hardness, and consumer acceptability. The PM was incorporated into ice creams prepared using vegan yogurt and analyzed for attributes including apparent viscosity, flow index (n), yield stress, viscoelastic constants (G’ and G’’), overrun, melt rate, color parameters (L*, a*, b*), and hardness using texture profile analysis, followed by sensory evaluation conducted by consumers on taste, texture, appearance, and overall acceptability. All formulations exhibited non-Newtonian behavior ($n < 1$); PM at 0.75% had the highest viscosity value (585.3 mPa·s) and least melting (1.20 g/g) and initial dripped time (67.2 min), indicating good thermal stability; PM at 0.5% achieved the highest consumer acceptability score (7.58), comparable to GG (7.61), demonstrating its efficiency as a natural stabilizer, while CAR and PM 0.75% reduced ratings owing to greater hardness and color variation. This study effectively demonstrated the efficiency of natural plant

mucilage extracts as stabilizers in vegan ice cream formulations and has direct relevance to the present study because gond katira (tragacanth gum), which is chemically similar to natural plant mucilage extract, has similar binding capacity with water and stabilization properties and would be expected to produce similar results.

**Acar *et al.* (2025)** [83] developed and characterized vegan ice creams formulated using red kidney bean milk (RKBM), either with or without the thermophilic probiotic Bacillus coagulans ATCC 7050, comparing it to dairy ice creams to evaluate its technological feasibility and potential benefits. Four types of ice creams were made: control (dairy ice cream without the probiotic, C); dairy ice cream containing the probiotic, CP; vegan ice cream formulated from RKBM without the probiotic, R; and vegan ice cream formulated from RKBM containing the probiotic, RP. Fermentation or ripening took place before freezing and hardening. The ice creams were evaluated for their dry matter, protein, pH, acid content, color (L*, a*, b*), overrun, hardness, antioxidant capacity (TEAC), TPC, and viability of the probiotic (CFU/g). The dry matter was in the range of 33.80% to 35.54%, while the protein content was in the range of 2.05% to 2.20%, without any significant differences being observed in vegan and dairy ice cream; however, the vegan samples were lighter, but had greater overrun (36.00-37.50%) than the dairy samples, with considerably higher antioxidant capacity (5224-6148 μM TEAC/g compared to dairy) and TPC (72.98-184.75 mg GAE/100g). The viability of the probiotics in RP remained functionally significant (6.93 $\log_{10}$ CFU/g after freezing); however, the vegan samples were rated lower in terms of their sensory properties than the dairy samples. From the findings of this research, it is clear that legume milk or pseudocereal milk may be used to manufacture functional ice creams superior to dairy ice creams in terms of bioactivity and probiotic properties, thus proving our hypothesis of using amaranth milk as the protein base for this research.

**Caponio *et al.* (2025)** [84] investigated the incorporation of oat okara, a byproduct rich in proteins and fibers formed during the production process of oat milk, which is usually discarded, into the formulation of plant-based ice creams, with the objective of reducing food wastage as well as enhancing the nutritive, rheological, and sensory qualities of ice creams. Different quantities of oat okara were incorporated into the formulation of plant-based ice cream products, and their analysis was done for dietary fibers, protein, fat, and carbohydrates using the proximate method, as well as their rheological parameters including apparent viscosity and viscoelasticity. The addition of oat okara greatly increased the dietary fibre content across all levels of inclusion, designating the product as a functional food; rheological stability improved at moderate levels of okara, as the water-retaining polysaccharide fraction enhanced viscosity and structural stability, and melting resistance was correspondingly improved; however, elevated okara levels resulted in a somewhat gritty texture that diminished sensory appeal, while moderate inclusion levels retained acceptable ratings. The research confirmed the idea of employing underused plant by-products abundant in dietary fibre as sustainable and functional components in vegan ice cream, directly endorsing the use of jackfruit seed powder in this study — another underutilised by-product that possesses similar fibre-boosting and structural characteristics — to create a nutritionally improved plant-based frozen treat while aiding in waste minimization.

**Kiełczewska *et al.* (2025)** [85] Aims to determine the compatibility of physical and sensory attributes of three protein-based dairy ingredients — micellar casein concentrate (CN), buttermilk protein concentrate (BMP), and whey protein concentrate 80% protein (WPC 80) — as functional food additives in ice cream production. The rising consumer demand for high-protein frozen desserts and the paucity of literature

comparing protein interactions in ice cream composition were considered in the development of the experiments. Ice cream formulations were prepared using each ingredient in place of skimmed milk powder (SMP) in ice cream formulation, and its total protein content, lightness (L*), redness (a*), yellowness (b*), and consistency index were determined, while overrun, melting rate, hardness, and sensory properties of the final ice creams were measured. Among the mixtures, WPC 80 resulted in the darkest shade of ice cream and the lowest overrun values, showing the highest hardness and lowest fluffiness ratings in sensory tests due to the development of a dense three-dimensional β-lactoglobulin network that enhanced viscosity and limited air incorporation during freezing; CN and BMP produced ice creams with enhanced overrun and improved fluffiness ratings, with CN resulting in the lightest color, while BMP ice cream demonstrated comparable sensory evaluations to the SMP control at a lower cost; protein levels were significantly increased across all fortified formulations compared to the control, with no notable differences in melting rate found between CN and BMP treatments. The research determined that incorporating high-protein dairy preparations into ice cream can enhance its nutritional and functional aspects; however, the selection of protein preparation plays a crucial role in shaping the product's structure, texture, and sensory attributes. These results are closely related to the current research, as germinated amaranth protein—a plant-derived protein with a dominance of albumin and globulin—is expected to engage with the ice cream matrix similarly to these dairy products, making the management of texture and structure through stabiliser incorporation a crucial aspect of formulation.

**Cruz and Martinez-Monteagudo (2025)** [86] with the objective of carrying out systematic investigation on how modification in the ratio of whey protein to casein in ice creams will influence rheology, overrun, hardness, meltdown properties, and shape retention, with an objective of formulating guidelines for protein ratio in order to produce high protein

ice cream with improved physical properties. Five whey protein to casein ratios, which include 13:87, 20:80, 33:67, 45:55, and 54:46 were subjected to investigation using a controlled matrix composition of 4.5% total protein, 13% total fat, and 42% total solids where rheology tests for ice cream mix were carried out while meltdown properties, hardness (by texture profile analysis), and shape retention tests were conducted for resultant ice cream. The formulations with higher casein contents (13:87 and 20:80) had significantly high overrun values of 56.21% and 38.14%, and low hardness values of 52.13 N and 58.14 N, respectively, while those with high whey protein content gave high hardness value of 75.18 N and minimal overrun of 20.83%. It was observed that the 20:80 whey: casein formulation delayed the onset of meltdown by 1.8 times compared to 54:46 mixture, which indicates that ice cream containing more casein has the potential to retard melting and preserve its form. In rheology studies, it was found that mixtures with high casein content showed higher apparent viscosity and shear thinning properties, which were conducive to trapping air during the freezing process, making it light-textured in nature. From the study, it is evident that casein plays an essential structural role in ice cream, affecting both the amount of air trapped during the process and the resistance to melt of the final product. However, higher levels of whey protein, have a negative effect on the physical properties; these findings form an essential basis for understanding the problems expected to be encountered in the present study, which is based on an amaranth protein network matrix that does not have a micellar structure because of the absence of casein.

**Abou Ayana *et al.* (2025)** [87] aims to innovate a novel biofunctional ice cream from enzymatically treated oat milk as a non-animal dairy substitute facing the dual challenge of improving the physicochemical and nutritional qualities of oat milk, which exhibits problems with starch gelation and Maillard reactions when heated in conventional ways, as well as evaluating its efficacy in preserving Lactobacillus acidophilus

during frozen storage as a functional probiotic carrier. Oat milk was prepared via a distinctive cold water α-amylase enzymatic process devoid of heat treatment that successfully converted starch to glucose without gelation, browning, and β-glucan content reduction; the following were formulated to replace skimmed milk with oat milk in a ratio of 25%, 50%, 75%, and 100% and inoculated with L. acidophilus followed by testing for overrun, viscosity, firmness, rate of melting, color, total β-glucan, total phenolics, antioxidant power (DPPH) and probiotic counts (log CFU/g) at week 0, 4, 8, and 12 during frozen storage. The enzyme treatment successfully inhibited gelation and browning at all substitution percentages, leading to the creation of a stable oat milk suspension with similar viscosity, viability of L. acidophilus in the ice creams made from oat milk remained intact between 25 to 50% substitution level as the counts were always greater than the functionally viable limit of 106 CFU/g over a period of 12 weeks, while there was lower probiotic survival at 100% oat milk substitution level due to the absence of dairy proteins which are responsible for cryoprotective buffering effects; there was an increase in β-glucan level proportionately with increasing levels of oat milk; antioxidant capacity and total phenolic content were considerably more in ice creams produced using oat milk formulations; sensory evaluation indicated acceptable taste and texture attributes in formulations with 25 to 75% substitution level of oat milk. From the above results, it may be stated that enzyme-treated oat milk can serve as a good plant-based substrate to develop biofunctional ice cream by incorporating probiotics, and it is capable of delivering clinically relevant quantities of β-glucan.

**Bekiroglu *et al.* (2024)** [88] aimed to evaluate the effect of levels of xanthan gum and oat-based milk substitute (OBMS) on the rheology, overrun, melt resistance, texture, color attributes and sensory acceptance of a totally vegan frozen dessert, based on the increasing interest in oat milk as the base, as well as the role played by

hydrocolloids in solving the texture problems inherent in plant-based frozen desserts compared to dairy products. Vegan ice creams were prepared from the OBMS, containing various amounts of xanthan gum, and their apparent viscosity, flow behavior index, dynamic oscillatory modulus (G', G''), overrun, time for first drip, L*, a*, b* values and hardness were determined using texture profile analysis, and sensory evaluation was done on their color, flavor, texture, creaminess and overall acceptance. All samples had non-Newtonian shear-thinning behavior, as characteristic of viscoelastic frozen dessert systems; higher levels of xanthan gum increased the values of apparent viscosity and elasticity modulus and lowered the overrun and first drip times, indicating higher stability and heat resistance; the β-glucan content in oat milk had a positive impact on viscosity and creaminess compared to control samples without OBMS, and in optimal amounts of xanthan gum, the sensory evaluation of creaminess and overall acceptability was equivalent to regular ice cream, while higher amounts led to excessively gummy consistency and lower flavor scores. The study showed that the use of a plant milk substitute based on oats, combined with the correct amount of natural hydrocolloid stabilizer, may lead to the development of vegan ice cream products with desirable characteristics. This information is relevant to the current study, as the same role may be expected from the gond katira (tragacanth gum), which has similar structure to the selected stabilizer and is also used in other plant milk-based ice cream products.

**Szydłowska *et al.* (2024)** [89] also conducted a narrative review in addition to their experimental study to fully explore the aspects of technology, microbiology, and sensory perception affecting the quality of probiotics in plant-based frozen desserts. This article tackles the growing preference of consumers towards plant-based desserts and functional foods with probiotic properties, while at the same time recognizing the lack of understanding about the mechanism of interactions between

probiotic microorganisms and plant-based matrices during production and preservation. The authors' narrative review incorporated literature findings with their own studies about plant-based frozen probiotic desserts, which included probiotic strain selection, survival rate under cold and acidic conditions, mechanisms of protection provided by prebiotics like inulin, FOS, GOS, and XOS, role of cryoprotectants, effects of fat content, protein sources, and type of sugar in developing ice crystals in plant-based probiotic matrices, and consumer sensory evaluation of plant-based probiotic frozen desserts. It is important to note that plant-based matrices were demonstrated to exhibit a range of advantages compared to dairy ones when used in probiotics, mainly because of low natural bacterial load that allows for better control over the fermentation process and higher amounts of polyphenols which help with antioxidant effects on probiotics. However, there were challenges associated with using plants, namely, reduced buffering ability and absence of milk proteins that function as cryoprotectant for probiotics. Adding prebiotics (such as inulin and FOS) increased viability of probiotics by 1–2 log CFU/g while simultaneously increasing texture due to increased viscosity and formation of gel; it is possible to achieve a sufficient concentration of probiotics at the minimum threshold of $10^6$ CFU/g that would make the product functional and therapeutic. The review indicated that frozen desserts containing prebiotics are a promising area of functional foods as sources for therapeutic doses of probiotics. These results explain the selection of FOS and resistant starch as prebiotic ingredients in the present formulation, as it is expected that these compounds will provide structural and functional roles in the formula.

**Limjaroen and Kijroongrojana (2024)** [90] with the aim of investigating the synergistic effect of fat and prebiotic (galactooligosaccharide [GOS] and fructooligosaccharide [FOS]) on the survival rate of Bifidobacterium longum TISTR 2195 in yogurt ice cream,

as well as studying the physical properties, rheological properties, and microstructure of the ice cream, in order to solve the problem of creating a formula with low fat content, high-quality physical properties, and high probiotic viability in frozen synbiotic ice cream. Yogurt ice cream was produced with two fat contents (2% and 10%), two types of prebiotics (GOS and FOS), at various concentrations, supplemented with B. longum TISTR 2195. Ice cream samples were analyzed for their apparent viscosity, flow behavior, dynamic oscillatory rheology, overrun, hardness, melting rate, color, and microstructure of ice crystals and air bubbles with the use of optical and electron microscopy, and probiotic viability through serial plate counts before and after in vitro gastrointestinal digestion. In all cases, FOS at its optimal concentration proved more efficient than GOS in sustaining the viability of probiotics for up to 12 weeks of freezing by minimizing the log CFU/g decrease while ensuring that the number of living cells remained above 106 CFU/g after the experiments; formulations with high-fat content showed superior dispersion of ice crystals and air cells, which contributed to high creaminess scores; FOS substantially increased the apparent viscosity of the ice creams, contributing to low ice crystal recrystallization and overrun stability; formulations with low-fat content had poor textural quality owing to their coarse microstructures and greater hardness, although the viability of probiotics was equally maintained with FOS included.

**Gorman *et al.* (2023)** [91] determined whether consumer sensory perceptions of plant-based frozen desserts differed from that of traditional dairy ice creams under blind tests versus non-blind conditions, with ingredient lists available. Vanilla-flavored frozen desserts currently on the market consisting of one traditional dairy ice cream, one frozen dairy dessert (FDD), and three plant-based varieties made from coconut, cashew, and soy base were subjected to consumer panel test using two methods, namely blind testing and testing in which information about

ingredients was revealed fully; overall liking, flavor, and texture of products were evaluated via hedonic scales of 9 points, while check-all-that-apply (CATA) sensory profiling was used to find out the descriptors most characteristic of particular products. In blind testing conditions, dairy ice cream was highly preferred over other plant-based products in terms of its flavor, texture, and overall liking. Plant-based frozen desserts were characterized by descriptors such as sandiness, watery sensation in the mouth, unpleasant flavor, and lack of lubrication, all attributed to inadequate emulsification of plant proteins, the lack of partially combined fatty structures, and presence of native volatiles from plants that impart beany/grassy flavors; the unveiling of ingredients resulted in slight improvements in consumer ratings of plant products, but the rankings remained the same, which implies that there was some effect of positive perception of health and environmental benefits on the perception of bad sensory qualities, but it was not enough; the frozen dessert with soy proteins got the worst rating in all scenarios because of the overwhelming beany flavor. In summary, the work demonstrated that intrinsic problems with texture and flavor, primarily the grittiness of the product and the unpleasant taste of proteins, is what prevents consumers from accepting plant-based frozen desserts. This research provides an important basis for selecting ingredients for the current work; namely, the use of germinated amaranth milk (lower anti-nutrients/off-flavors), gond katira (texturizer), and monk fruit sweetener (counteracts cereal aftertaste).

**Hidas *et al.* (2023)** [92] explored the influence of distinct varieties of plant proteins (pea, soy, rice) together with guar gum and microbial transglutaminase (MTGase) on the physical and textural attributes of vegan ice cream prepared from hemp drink as a result of challenges with overrun, rapid melting, and poor textural qualities that characterize plant-based frozen desserts when compared with their dairy counterparts. Vegan ice creams based on hemp drink were prepared

using diverse types of plant proteins supplemented with guar gum and MTGase following conventional ice cream preparation techniques. The test samples were analyzed for their apparent viscosity, overrun, hardness, melting rates, elastic modulus (G'), and viscous modulus (G''), alongside their sensory evaluation of texture and taste. Shear-thinning behaviour was observed in all samples, where the addition of guar gum improved structure stability and resulted in higher viscosity, and the addition of MTGase caused protein cross-linking, thereby resulting in a tougher texture; the formulations containing 25% hemp drink along with optimal protein and stabilizer content received the best sensory scores, whereas the addition of more hemp drink increased phenolic content and antioxidant potential but had a faster melt rate due to the presence of high amounts of polyunsaturated fatty acids in the hemp oil. It was evident that the importance of selecting an appropriate stabilizer for plant-based ice creams needed to be emphasized to ensure desirable physical attributes, and the direct recommendation was the use of gond katira (tragacanth gum), which could play a similar role as guar gum for the development of amaranth milk vegan ice cream.

**Jan *et al.* (2023)** [93] carried out an extensive narrative review to compile existing information on the nutritional profile, antinutritional components, health-enhancing properties, and various uses in food, medicine, and cosmetics for amaranth and quinoa, intending to encourage their broader use as functional food ingredients and nutraceuticals in conventional food systems. An extensive search in PubMed, Scopus, and Web of Science was conducted concerning the study of composition analysis and the effect of different processing techniques, including germination, fermentation, and extrusion, which have an influence on reducing antinutritional components while enhancing their bioavailability and health benefits. Amaranth grain has been identified to possess 15–16% protein with a comprehensive amino acid profile that is notably better than traditional cereals — particularly

elevated in lysine (55–65 mg/g protein) and sulfur-containing amino acids. Its lipid content (6–9%) includes as much as 83% unsaturated fatty acids and remarkably high squalene levels (up to 11% of total lipids), offering cholesterol-lowering and skin-protective benefits. Bioactive phenolic compounds such as quercetin, kaempferol, rutin, ferulic acid, and gallic acid provide antioxidant, anti-inflammatory, antidiabetic (inhibition of DPP-IV and alpha-glucosidase), antihypertensive (ACE inhibition), and hypocholesterolaemic effects. The antinutrients phytate (2.9–7.9 g/kg) and saponins (0.9–4.91 mg/kg) decreased significantly through germination, which simultaneously enhanced protein availability by 8%, total energy by 11%, and reduced resistant starch by 70%, improving both digestibility and bioavailability. The review definitively demonstrated that germinated amaranth is a nutritionally enhanced pseudocereal, offering significantly improved protein bioavailability and lower antinutrient levels. This directly supports the choice of germination pre-treatment for amaranth as the main milk base in this study, scientifically confirming germination as the best processing method to enhance the nutritional quality and functional efficacy of the amaranth milk utilized in this formulation.

**Kowalczyk *et al.* (2022)** [94] studied whether adding fibres like inulin and apple fibre to sheep milk ice cream can help protect five different types of good bacteria during digestion. This study was done because sheep milk is nutritious and prebiotic fibres are good at surviving stomach digestion and helping good bacteria grow. They made four types of ice cream using sheep milk: one without any added fibre, one with 4% inulin, one with a mix of 2.5% inulin and 1.5% apple fibre and one with 4% apple fibre. Each type was then added with five different good bacteria. Lacticaseibacillus casei 431, acidophilus LA-5, Lacticaseibacillus paracasei L-26, Lacticaseibacillus rhamnosus and Bifidobacterium animalis ssp. Lactis BB-12. They then tested how well these bacteria survived in a digestive system. The results showed that

adding 4% inulin helped Bifidobacterium animalis BB-12 survive better in the stomach with only 16.7% dying off. In the intestine 4% inulin also helped Lacticaseibacillus rhamnosus and Bifidobacterium animalis BB-12 survive by 10.8% and 22% respectively. Adding 4% apple fibre helped Lacticaseibacillus casei and Lacticaseibacillus paracasei survive by 7.86-11.26%. Most of the bacteria in the ice cream survived well with numbers between 6.11 and 8.88 log cfu/g in the intestine. This is enough to help people feel better. The study found that prebiotic fibres, inulin really helped the good bacteria survive in the ice cream by forming a protective layer during digestion. Thus, addition of dietary fibers such as inulin, FOS and resistant starches may be beneficial in maintaining a healthy balance of intestinal microflora in the gut.

**Narala *et al.* (2022)**[95] examined the application of inulin to the fat content of vegan ice cream based on pea protein. The aim was to reduce saturated fats while maintaining desirable organoleptic characteristics and ensuring consumer satisfaction. The popularity of frozen products based on pea protein rises every day. Recipes for producing vegan ice creams were developed by using pea protein concentrate as the major source of protein. Fat was gradually substituted for various amounts of inulin. Samples underwent the processes such as mixing, pasteurizing, homogenizing, aging and freezing. The following tests were performed: hardness, overrun, resistance to melting by analytical and melt-down methods. Inulin-based ice cream possessed overrun. It had creamier and smoother characteristics. It melted slower than control samples. This happened due to the formation of a gelling network. The network functioned similarly to globules in the structure of ice cream. However, acceptability and texture in samples with inulin were significantly better than in the control samples. Therefore, inulin was proven to be an additive. Its role could be in fat replacement in ice cream serving as a prebiotic fiber. In conclusion, the study proved that inulin is able to replace fat in vegan ice cream.

**Narala *et al.* (2022)** [96] extended their research to explore the possibility of using inulin in producing vegan yoghurt. Their objective was to produce a frozen vegan yoghurt ice cream based on pea protein which could sustain the growth of the bacteria within it after freezing. It was important that it should also be tasty and have a certain texture without containing dairy fat. Vegan yoghurt ice cream containing pea protein was produced. Inulin was added to it in various concentrations. Then, vegan yoghurt cultures were added to it. Observations were made for the growth of bacteria in various stages of production. At each stage, observations were made regarding its acidity, viscosity, and hardness. Also, sensory assessment was made by taking views of individuals as regards its creamy nature and mouth feel. There was an increase in good bacteria with the addition of inulin in the ice cream. It also made the ice cream smoother and creamy. Ice cream with inulin added to it was also more appreciated by people because it tasted better. This research demonstrated how beneficial inulin was for vegan products. It promotes the growth of good bacteria and enhances texture. Therefore, there is no doubt that inulin along with FOS and starch of foxnut and jack fruit seed powder should be used in making vegan ice cream. Not only will they improve gut health, but their usage in ice cream will also enhance its quality and taste.

**Velotto *et al.* (2021)** [97] aiming to reduce sugar content and increase the nutritional value of dairy and vegan handmade ice cream, sugar was replaced with stevia, a zero-calorie natural sweetener made from Stevia rebaudiana steviol glycosides, and chia seeds were included for their omega-3 fatty acid, fiber, protein content, and mucilage. Two different types of ice cream formulas were developed: dairy-based and vegan — where stevia substituted sucrose at suitable levels and chia seeds were added at different rates; the nutritional profile, including sugar, fiber, protein, and omega-3 fatty acid content, was evaluated along with

physicochemical aspects (texture, color, melting characteristics) and consumer preferences through sensory evaluation. The employment of stevia resulted in significantly decreased amounts of total sugar and total calories without affecting sensory characteristics at proper concentrations of stevia, whereas the addition of chia seeds considerably increased total dietary fiber and omega-3 contents. Both types of ice creams contained the same textural and sensory properties compared to regular ice cream; additionally, it was found that vegan stevia-based and chia-added samples had similar sensory scores to those obtained from dairy samples, which supported the possibility of compensating for the absence of fat content in vegan products through functional ingredients. The findings revealed the effectiveness of using natural zero-calorie sweeteners and functional seeds in improving health value without compromising sensory attributes in vegan ice cream, thereby providing scientific support for choosing monk fruit sweetener (Siraitia grosvenorii) as a natural, non-caloric, and intense sweetener.

**Lumbantobing *et al.* (2019)** [98] attempted to use jackfruit seeds that are generally discarded to produce vegan ice cream. They constitute 90 percent of the waste generated during the production of jackfruit seeds. Researchers utilized the seeds as the source of plant milk. Jackfruit seeds contain a variety of nutrients including starch, protein, fiber, and essential minerals. They subjected the seeds to washing, boiling, and peeling before transforming them into milk through milling and filtration processes. Researchers produced three different types of ice cream; one served as the control while the other two utilized additives to assist in mixing the constituents. They conducted tests on the ice cream to determine its nutritional composition, including fiber, carbohydrates, fats, and proteins. They also evaluated its physical appearance and texture. In addition, they conducted a sensory evaluation by asking 39 individuals to taste the ice cream. They conducted a statistical analysis of the findings. The ice cream obtained from the jackfruit seeds contained

fiber. Moreover, it did not contain high amounts of fat compared to ordinary ice cream. However, one variant of the ice cream that had additives such as lecithin was more viscous than the others. From the results of this study, it was found out that the seeds of the jackfruit can actually be made into milk which can then be used for creating vegan ice creams that are both low-fat and high in fibre content.

# CHAPTER-III

# MATERIALS & METHODS

# MATERIALS AND METHODS

The current research involved an experimental approach, with the objective of developing and testing prebiotic ice cream made from plant sources, with high protein content, prepared with germinated amaranth milk, makhana, jackfruit seed powder, gond katira, and monk fruit sweetener. The study was conducted at NIMS University, Rajasthan, Jaipur over a period of six months. The methodology was carried out in six structured phases covering market survey, procurement and processing of raw materials, product development and standardization, sensory evaluation, proximate and physicochemical analysis, antioxidant potential assessment, shelf-life estimation, and cost and packaging analysis.

## 3.2 STUDY LOCATION

The largest city in Rajasthan, India, is Jaipur, historically known as "Jeypore". The capital of Rajasthan is Jaipur and the specific area of the study is Nims University, Rajasthan, Jaipur, which is basically located on Jaipur- Delhi highway (NH-11C). The study was conducted at the cookery lab of Nims University, Rajasthan, Jaipur.

**Fig 4-** Location of the study

## 3.1 STUDY DESIGN

The study was interventional , as it involved the development of food product. This chapter is a comprehensive research design overview and an outline of the steps accomplished in the methodology throughout the study. For ease of comprehension, it is divided into seven phases from the preliminary step to the final step. Each phase is described in detail, including methods, techniques, and procedures that were utilized to fulfil the objectives of the research. This study was conducted in the following seven phases-

**PHASE I**: MARKET SURVEY OF COMMERCIALLY AVAILABLE CHOCOLATE TRUFFLES

**PHASE II**: PROCUREMENT AND PROCESSING OF RAW MATERIAL FROM ONLINE PLATFORM AND LOCAL MARKET

**PHASE III**: STANDARDIZATION AND FORMULATION OF MILLET AND HERB FUNCTIONAL CHOCOLATE TRUFFLES

**PHASE IV**- SENSORY EVALUATION OF THE FORMULATIONS AND STATISTICAL ANALYSIS OF DEVELOPED VARIATIONS

**PHASE V**: PROXIMATE COMPOSITION, PHYSICO- CHEMICAL PARAMETES AND SHELF-LIFE ANALYSIS (AOAC 2023) OF THE MOST ACCEPTABLE PRODUCT

**PHASE VI**: DEVELOPMENT OF PACKAGING, FOOD LABEL, AND COST ANALYSIS OF THE PRODUCT

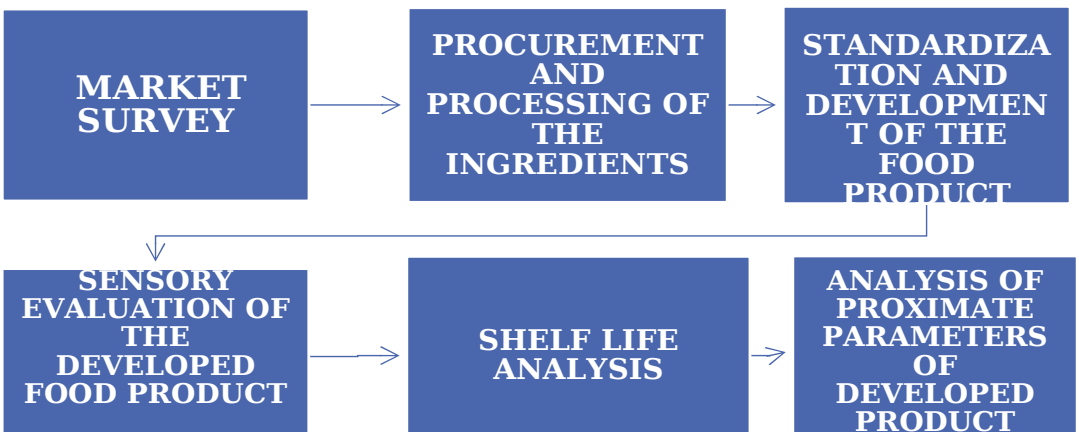

MARKET SURVEY
PROCUREMENT AND PROCESSING OF THE INGREDIENTS
STANDARDIZATION AND DEVELOPMENT OF THE FOOD PRODUCT
SENSORY EVALUATION OF THE DEVELOPED FOOD PRODUCT
SHELF LIFE ANALYSIS
ANALYSIS OF PROXIMATE PARAMETERS OF DEVELOPED PRODUCT

**Fig 3.1** Flow chart of methodology

### 3.3 STUDY PERIOD

Study period for research was 6 months. As the study design was experimental in nature, it took 6 months to complete this study followed with market survey, food product development, data collection, statistical analysis and results interpretation.

### 3.4 Study population

The study population included individuals above 18 years of age.

### 3.5 INCLUSION CRITERIA

- Semi- trained panelists above 18 years of age were included in this study as sensory evaluation was conducted for the developed formulation.

### 3.6 EXCLUSION CRITERIA

- All the individuals except the semi- trained panelists.

## 3.7 Statistical analysis

Using Excel, the data that was obtained was analyzed. To summarize the data, descriptive statistics were computed, including percentages, means, and standard deviations. Tables, graphs, and charts were used to portray the data in a way that was easy to understand.

## METHODOLOGY

### PHASE I – Market Survey of Commercially Available Plant-Based and Functional Ice Creams

### 3.1 Market Survey

Understanding the market is crucial before creating any new food product. To do this a market survey was done to find out what consumers want and need. The survey looked at what ingredients are popular what nutrients are missing in existing products and how aware people are of plant-based and functional ice creams. The survey was done in a way checking out retail stores, supermarkets and online platforms like

Amazon and BigBasket. It also looked at brand websites and product information that has been published. Information about the name of the product, the brand, the ingredients, nutritional information, and cost were all gathered. This information was gathered about vegan, high protein, and sugar-free varieties of ice cream which are available in the market in India and abroad.

## PHASE II – Procurement and Processing of Raw Materials

### 3.2 Study Area

The experiment was carried out in the cookery laboratory of NIMS University, Rajasthan, Jaipur. All processes related to product development, standardization, and sensory evaluation were performed in the same place. Laboratory tests such as proximate analysis, physicochemical analysis, and antioxidant activity tests were done in a recognized laboratory through standard analytical equipment.

### 3.3 Procurement of Raw Materials

All raw materials used in this study were procured from reliable local markets in Jaipur and verified online retail platforms (Amazon India, Confect). The following ingredients were sourced: amaranth grains (for germinated amaranth milk), foxnut (makhana), jackfruit seeds (for jackfruit seed powder), tragacanth gum (gond katira), monk fruit sweetener, almond butter, cocoa powder, walnuts, almonds, cashews, and dark chocolate (70% cocoa preferred). Before each batch of raw materials could be used, it was checked for freshness, cleanliness, and absence of spoilage or contamination. The size of each batch was kept standardized during the entire experiment.

### 3.4 Processing of Raw Materials

Each ingredient was pre-processed individually before being incorporated into the ice cream formulation. The processing methods are described below.

### 3.4.1 Processing of Amaranth Seeds for Germinated Amaranth Milk

The amaranth germination process was done to increase protein bioavailability, eliminate antinutrients, and improve the nutritional content of the amaranth milk. This procedure involved the following processes:

- Manual selection and cleaning of the amaranth grains by removing foreign materials, damaged kernels, and other extraneous substances.
- Three or four washes with clean water followed by soaking the grains in distilled water for 8–12 hours at room temperature.
- Complete removal of the excess water after soaking, followed by spreading the grains uniformly on the wet muslin cloth or filter paper in germination trays.
- Covering of the germination trays with wet muslin cloth and incubation in a dark place at a temperature of 28–30°C for 24–48 hours.
- Washing the sprouted kernels with clean water to eliminate surface microorganisms.
- Mixing the sprouts with distilled water in a high-speed blender, followed by filtration of the mixture through a double-layered wet muslin cloth to extract the amaranth milk.
- Pasteurisation of the milk at 72°C for 15–20 seconds using a water bath and cooling to 4°C for storage purposes.

**Cleaning of amaranth grains**

↓

**Soaking (8–12 hours)**

↓

**Spreading on moist filter paper/muslin**

↓

**Covering and incubation (28–30°C, 24–48 hrs)**

↓

**Germination of grains**

↓

**Grinding with distilled water**

↓

**Filtering to obtain amaranth milk**

↓

**Pasteurization (72°C, 15–20 sec)**

↓

**Cooling and storage at 4°C**

**Figure 6 – Flowchart of Germinated Amaranth Milk Processing**

[99]

### 3.4.2 Processing of Foxnut (Makhana)

- Foxnuts were washed thoroughly to eliminate any dust or debris.
- Foxnuts were roasted in a dry iron skillet at medium heat until they puff up and become crispy.
- Foxnuts were cooled down to room temperature and then crushed into powder for some variations of the formulation, while others used the whole nut.

### 3.4.3 Processing of Jackfruit Seeds for Jackfruit Seed Powder

- Seeds of fresh jackfruits were obtained and washed in running water, then boiled in water for 15-20 minutes to make them soft and easy to peel off from their outer coating.
- The white outer layer of the seed was stripped off, and then the inner part of the seed was cut into very small pieces.
- These pieces were dried in hot air oven at 50-60°C until a steady weight was attained.
- The dried samples were powdered using a laboratory grinding machine, which was then sieved through an 80-mesh sieve.
- The powder samples were stored in sealed containers at room temperature.

### 3.4.4 Processing of Gond Katira (Tragacanth Gum)

- Flakes of gond katira with good quality were chosen and examined for any signs of discoloration or adulteration.
- They were allowed to soak in distilled water for 12 to 24 hours at room temperature.
- The gel was agitated to break down the lumps, resulting in a smooth texture.

- In case of any undissolved particles in the gel, it was passed through a sieve prior to its addition.
- The hydrated gum gel was then incorporated into the ice cream mixture during the stabiliser addition step, prior to pasteurisation.

### 3.4.5 Processing of Almonds and Cashews

- Almonds and cashews were soaked in water overnight to make them soft as well as eliminate anti-nutrients.
- Almonds were de-skinned after being soaked to remove the brown skin.
- Afterwards, both almonds and cashews were blended with little water to produce nut paste/milk that was used in making the mix.

### 3.4.6 Processing of Dark Chocolate

- The dark chocolate with 70% cocoa content was grated and then melted at 40-45 degrees Celsius with the use of a double boiler until smooth and without lumps.
- The molten chocolate was tempered where necessary, after which it was added to the ice cream mixture while stirring.

### 3.4.7 Processing of Walnuts

- The walnuts were roasted at temperatures between 100 °C and 120 °C for five to seven minutes.
- The walnuts were then cooled before being roughly cut into pieces.

### 3.4.8 Processing of Cocoa Powder

- Food grade cocoa powder was examined for any contamination, passed through a sieve of small mesh size for homogenous particle size, and dry roasted if necessary to improve flavor.
- The treated cocoa powder was then packed into a sealed container for later use.

### 3.4.9 Processing of Almond Butter

- The raw almonds were washed, soaked in water for 6 to 8 hours, and shelled.
- After roasting the shelled almonds lightly, the product was cooled down and processed in a food processor till it became almond butter.
- The almond butter was kept refrigerated until it was mixed into the ice cream mixture.

### 3.4.10 Processing of Monk Fruit Sweetener

- An industrially produced food grade sweetener made from monk fruit was selected and ensured to be free from any impurity and moisture lump formation.
- The sweetener was dissolved in a little amount of warm water in order to have an even distribution in the ice cream mixture.
- The dissolved solution was stored in a sterile airtight container.

## PHASE III – Standardization and Development of the Vegan Ice Cream

## 3.5 Standardization of the Product

Standardization is defined as the process where the best possible combination of ingredients along with processing parameters to obtain an acceptable product in respect of flavor, texture, color, and nutrient content is determined systematically. In this experiment, standardization was done via conducting a pilot test and formulating several sets of variations that were then tested by a sensory panel.

### 3.5.1 Pilot Study

The first test was conducted as a pilot test by applying a trial and error method using very small batches (200 grams per batch) to determine the feasibility of the formulation and observe the overall flavor, texture, color, and aroma of the base ice cream

### 3.5.2 Development of Formulation Variations

Four formulations of vegan ice cream were made – a control one and three variations AAA, BBB, and CCC – by changing the ratio between the two functional ingredients, cashew and gond katira, but keeping the same ratio of base ingredients like germinated amaranth milk, monk fruit sweetener, and others. It helped compare how different proportions affected the quality and nutrient content of the product. Control preparation was made based on standard ratio, which was determined during a pilot study; AAA, BBB, and CCC had various proportions of

makhana, jackfruit seed flour, and nut pastes to select the optimal one. The formula table is provided in Table 3.

**Table 3 : Formulation Chart for Vegan Ice Cream Variations (per 500-515 g base)**

| Ingredient | AAA | BBB | CCC |
|---|---|---|---|
| Germinated Amaranth Milk (ml) | 250ml | 250ml | 250ml |
| Foxnut powder (g) | 10g | 10g | 10g |
| Jackfruit seed powder (g) | 8g | 8g | 8g |
| Tragacanth Gum (hydrated gel, g) | 35g | 45g | 55g |
| Monk fruit sweetener (g) | 7g | 7g | 7g |
| Almond butter (g) | 25g | 25g | 25g |
| Thick coconut milk (ml) | 45ml | 45ml | 45ml |
| Dark chocolate (g) | 25g | 25g | 25g |
| Cocoa powder (g) | 10g | 10g | 10g |
| Cashews (g) | 70g | 85g | 55g |
| Vanilla extract | 3.75 ml | 3.75 ml | 3.75 ml |

### 3.5.3 Ice Cream Preparation Method

The ice cream mix was made based on the normal procedure of production but with some adjustments in view of its special composition. This is as explained in the steps outlined below and also in Figure 7.

- Accurate measurements were taken for all the raw materials involved in the production of the ice cream using a measuring balance.
- The base liquid chosen for the production of this particular ice cream was the germinated amaranth milk. This liquid was combined with nut pastes (almonds, cashews) to form a homogeneous base mixture.

- The makhana and jackfruit seed powders were added to the base mixture.
- The gond katira gel was used as the stabiliser in the mixture and blended properly to ensure that no lumps formed.
- Cocoa powder/dark chocolate was added to the mixture.
- The monk fruit solution was also added and blended until it was evenly distributed throughout the mixture.
- The entire mixture was heated at 72°C for 15-20 seconds before cooling to 4°C.
- The mixture was homogenized using a blender at moderate speeds.
- The homogenised mix was aged at 4°C for 6–12 hours to allow the stabiliser to fully hydrate and develop proper viscosity.
- The aged mix was frozen in a domestic ice cream maker (churning) at −18°C to −20°C until a soft-serve consistency was achieved, incorporating approximately 30–40% overrun.
- The semi-frozen product was transferred to labelled airtight food-grade containers and hardened at −18°C to −20°C for 6–8 hours before evaluation.

**Selection and cleaning of raw ingredients**

↓

**Processing of individual ingredients (as above)**

↓

**Preparation of amaranth milk (base)**

↓

**Incorporation of nut pastes (almonds, cashews, walnuts)**

↓

**Addition of makhana powder and jackfruit seed powder**

↓

**Hydration of tragacanth gum (stabilizer) and incorporation**

↓

**Melting and mixing of dark chocolate**

↓

**Blending all components into smooth mixture**

↓

**Pasteurization at 72°C for 15–20 seconds**

↓

**Cooling to 4°C**

↓

**Homogenization for uniform texture**

↓

**Aging of mix at 4°C for 6–12 hours**

↓

**Freezing with incorporation of air (overrun)**

↓

**Hardening at -18°C to -20°C**

↓

**Storage at -18°C until consumption**

**Figure 7 – Flowchart of ice cream preparation process**

## PHASE IV – Sensory Evaluation of the Developed Product

### 3.6 Sensory Evaluation

Sensory evaluation is an important scientific tool used to measure how human senses of taste, smell, appearance, and texture perceive a food product. It occupies an important position in the determination of consumer acceptability, in addition to ensuring that the developed ice cream achieves its nutrition goals and is also palatable to eat. In the current study, two successive sensory analyses were carried out.

### 3.6.1 Sensitivity Threshold Test

Prior to conducting any sensory evaluation tests, there was a test conducted to determine suitable panelists who were able to distinguish between tastes and textures.The test was conducted among 30 volunteers from NIMS University who had no known taste-related disorders or conditions. Sugar solutions of varying concentrations were prepared and presented to participants in random order. Participants were instructed to rinse their mouth with plain water before tasting each sample. Each participant recorded the level of sweetness detected for each sample using the sensitivity threshold proforma (Annexure 1). Results were statistically analysed using mean and standard deviation. Panelists who demonstrated adequate sensitivity were selected for the nine-point hedonic test.

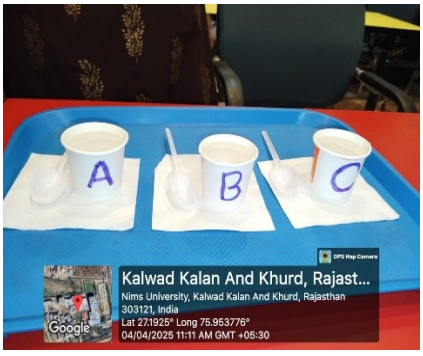
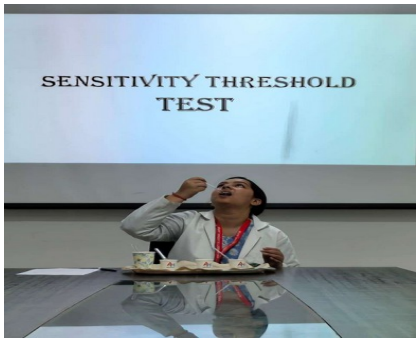



**Figure 8 – Sugar solution with varying concentrations**




**Panelists performing the sensitivity threshold test**

### 3.6.2 Nine-Point Hedonic Rating Scale

The four formulations — one control (C) and three variations (AAA, BBB, CCC) — were evaluated by a panel of 15 semi-trained individuals selected through the sensitivity threshold test. The study included participants who were above 18 years of age, in good health, free from colds, allergies, or taste disorders, and who voluntarily consented to participate. Individuals with known food allergies to nuts or seeds were excluded from the evaluation. Informed consent was obtained from all participants before the test was conducted.

Ice cream samples were served in pre-chilled, opaque paper cups at the standard serving temperature (−12°C to −14°C). Samples were coded with single-letter codes (C, AAA, BBB, CCC) and presented to panelists in randomised order on a tray along with a glass of plain water at room temperature for palate cleansing between samples. Panelists were seated in a well-ventilated room with adequate lighting and evaluated each sample independently.

(i)

(ii)

(iii)

**Figure 10 - ( i ) Developed product with control (ii) ,( iii ) Selected panelists performing the sensory evaluation,**

## 3.7 Statistical Analysis

All data collected during the study were statistically analysed using SPSS version 23.0 software and Microsoft Excel. Descriptive statistics, including mean and standard deviation (SD), were calculated for all sensory and analytical parameters. One-way ANOVA was applied to determine significant differences among the formulations, and post-hoc Tukey's test was used for pairwise comparison where applicable. A p-value of less than 0.05 was considered statistically significant. Results were presented in the form of tables and bar graphs for clear interpretation.

### 3.7.1 Mean

The mean was determined by taking the arithmetic average of all the observations recorded for each variable. It was used to show the central value of the sensory and analytical measurements collected.

### 3.7.2 Standard Deviation

The standard deviation (SD) was computed to measure the degree of variation or spread of the values from the mean using the standard formula in Microsoft Excel and SPSS.

## PHASE V – Proximate, Physicochemical, and Antioxidant Analysis

## 3.8 Proximate Analysis of the Developed Product

The most acceptably rated formulation, as determined by sensory evaluation, was selected for detailed nutritional analysis. Proximate composition was determined to assess the basic nutritional profile of the developed ice cream. All analyses were performed in triplicate, and results are expressed as mean ± standard deviation. The methods used are listed in Table 4.

**Table 4 : Proximate Analysis Parameters and Methods**

| Parameter | Method | Reference |
|---|---|---|
| Moisture content | Hot air oven method | AOAC (2023) |

| | | |
|---|---|---|
| Total protein | Kjeldahl method | AOAC (2023) |
| Total fat | Soxhlet extraction method | AOAC (2023) |
| Total carbohydrate | By difference (100 − protein − fat − ash − moisture) | AOAC (2023) |
| Crude fibre | McCleary acid–alkali digestion method | AOAC (2023) |
| Total ash | Muffle furnace incineration at 550°C | AOAC (2023) |
| Total solids | Gravimetric method | AOAC (2023) |
| Total energy | Atwater conversion factors | AOAC (2023) |

### 3.8.1 Determination of Moisture Content

The moisture content was measured by the hot air oven drying technique, as described by AOAC (2023). Porcelain crucibles were dried in an oven at 100°C for 30 min, cooled in a desiccator, and weighed as W1. A certain amount of the sample was weighed in each crucible as W2. The crucible along with the sample was dried at 100°C for 4-6 hr, cooled in a desiccator, and repeatedly weighed till a constant weight was attained as W3. Moisture content was calculated by using the following equation:

$$\text{Moisture \%} = [(W2 - W3) / (W2 - W1)] \times 100$$

### 3.8.2 Determination of Total Protein

Protein analysis was done through the Micro-Kjeldahl method as described by AOAC (2023). It consists of the following stages: digestion, distillation, and titration.

**Digestion:** About 0.5g of the sample was placed into the micro-kjeldahl digestion tube together with the catalyst of 5 parts of potassium sulphate and 1 part of cupric sulphate. The sample was then subjected to heat in the digestion apparatus until it became clear and colourless, indicating complete oxidation of the nitrogen present.

**Distillation:** After digestion, the sample was placed into the distillation apparatus and then 20 ml of 40% solution of sodium hydroxide was added. The ammonia gas released in the process is collected in a 100ml conical flask containing 4% boric acid and methyl red indicator of 2–3 drops.

**Titration:** Titration involved titration of the ammonia in boric acid using 0.1N hydrochloric acid. Colour change from green to pink marked the endpoint. The percentage nitrogen and crude protein were determined from:

$$\textbf{\textit{Nitrogen \% = [14 × N (HCl) × V (sample − blank) × 100] / Weight of sample (g)}}$$

$$\textbf{\textit{Protein \% = Nitrogen \% × 6.25}}$$

### 3.8.3 Determination of Total Fat

The percentage of fat in samples was obtained using Soxhlet extraction method (AOAC, 2023). Pre-weighted thimbles with 1g of the dried sample were added into the Soxhlet extraction apparatus along with petroleum ether (BP 60-80°C) in aluminium cans. The procedure was repeated until a drop speed of 2-3 drops per second was obtained within 2-3 hours. The aluminium cans were then dried at 100°C and their weight recorded after cooling to constant weight. The calculation of fat percentage was done by:

$$\textbf{\textit{Fat \% = [(W3 − W2) / W1] × 100}}$$

Where W1 = weight of sample, W2 = weight of empty canister, W3 = weight of canister with extracted fat.

### 3.8.4 Determination of Total Ash

The ash content analysis involved the use of a muffle furnace method (AOAC, 2023). In this procedure, 2 grams of the sample was put in a porcelain crucible, which was heated at 550°C for six hours until only ash remains white or grey in color. The ash content was computed using the formula:

$$\textbf{\textit{Ash \% = (Weight of ash / Weight of sample) × 100}}$$

### 3.8.5 Determination of Crude Fibre

Crude fibre was determined using acid-alkali wash procedure (McCleary method, AOAC 2023). This involved digesting a 2.5 g portion with hot concentrated sulphuric acid (200 ml) for 30 minutes. The material was then extracted with distilled hot water, boiled with hot concentrated sodium hydroxide (200 ml) for 30 minutes, washed with distilled hot water and 10 ml of alcohol and dried at 105oC to constant weight and finally, the crude fiber was incinerated in muffle furnace for 30 minutes at 600 oC. Crude fiber was determined as the difference between dry weight (W1) and the weight of ash (W2).

$$\textbf{\textit{Crude Fibre \% = [(W1 − W2) / Weight of sample] × 100}}$$

### 3.8.6 Determination of Total Carbohydrate

Total carbohydrate was calculated by the difference method (AOAC, 2023) using the formula:

$$\textbf{\textit{Carbohydrate \% = 100 − (Moisture \% + Protein \% + Fat \% + Ash \% + Crude Fibre \%)}}$$

### 3.8.7 Estimation of Total Energy

The energy value of the developed ice cream was calculated from the macronutrient composition using Atwater's conversion factors:

***Energy (kcal) = [Protein (g) × 4] + [Carbohydrate (g) × 4] + [Fat (g) × 9]***

## 3.9 Physicochemical Properties of the Product

The physicochemical properties include the physical and chemical quality attributes of ice cream, which affect the sensory appeal and stability of the product. The variables evaluated and their corresponding techniques are shown in Table 5.

**Table 5 : Physicochemical Analysis Parameters and Methods**

| Parameter | Method | Reference |
|---|---|---|
| pH | Digital pH meter | AOAC (2023) |
| Total Soluble Solids (TSS) | Hand/digital refractometer (°Brix) | AOAC (2023) |
| Overrun (%) | Weight–volume method | AOAC (2023) |
| Melting rate | Gravimetric melt-down method | AOAC (2023) |
| Colour analysis | Colorimeter / Hunter Lab (L*, a*, b*) | AOAC (2023) |

### 3.9.1 pH

The pH value of the most popular ice cream mixture was determined by a digital pH meter (Elico LI-127) that was previously calibrated. The probe was thoroughly washed after each measurement with distilled water. Measurements were performed three times and an average was calculated for each sample.

### 3.9.2 Total Soluble Solids (TSS)

Total soluble solids were measured using a hand or digital refractometer and expressed in degrees Brix (°Brix). A small quantity of the melted ice cream sample was placed on the prism of the refractometer, and the reading was noted in triplicate.

### 3.9.3 Overrun

Overrun is the difference between the volume of the ice cream produced and that of the mix, presented as a percentage. This is the volume of air mixed into the product while making it. Overrun was determined using the weight-to-volume ratio method:

$$\textbf{\textit{Overrun \% = [(Weight of mix − Weight of ice cream) / Weight of ice cream] × 100}}$$

### 3.9.4 Melting Rate

The procedure used to measure melting behavior involved the use of the gravimetric melt-down technique. Ice cream of known weight was placed on a metal wire grid that was suspended above a beaker. Melting rates were measured as the weight of the dripping fluid that was collected in the beaker after certain time intervals (every ten minutes over sixty minutes).

### 3.9.5 Colour Analysis

Color measurements were performed using a colorimeter with Hunter Lab color system parameters, which includes L* (lightness), a* (red/green value), and b* (yellow/blue value). Prior to measurement, calibration was done using white and black tiles.

## 3.10 Antioxidant Potential of the Developed Product

Antioxidant potential was evaluated to quantify the ability of the product to scavenge free radicals and assess its health-promoting value. The parameters and methods used are presented in Table 6.

**Table 6 : Antioxidant Analysis Parameters and Methods**

| Parameter | Method | Reference |
|---|---|---|

| | | |
|---|---|---|
| Total Phenolic Content (TPC) | Folin–Ciocalteu method, UV-Vis spectrophotometer | AOAC (2023) |
| DPPH Radical | DPPH assay at 517 nm, UV-Vis spectrophotometer | AOAC (2023) |

### 3.10.1 Total Phenolic Content (TPC)

The total phenolic content was measured using the Folin-Ciocalteu reagent colorimetric assay. An amount of ice cream extract was reacted with the Folin-Ciocalteu reagent along with the sodium carbonate solution. The absorbance was then recorded after a 30-minute incubation period in a spectrophotometer at 765 nm. The total phenolic content was presented in terms of gallic acid equivalent in mg/100g of sample.

### 3.10.2 DPPH Radical Scavenging Activity

The scavenging ability of the DPPH radical (2,2-diphenyl-1-picrylhydrazyl) was done according to the procedure described by Brand-Williams et al. A certain volume of the extract sample was introduced into a methanolic solution of DPPH (0.1 mM). After incubation for 30 minutes in darkness, the absorbance was read at 517 nm. Percentage inhibition was calculated using:

$$\% \text{ Inhibition} = [(\text{Absorbance of control} - \text{Absorbance of sample}) / \text{Absorbance of control}] \times 100$$

## PHASE VI – Shelf-Life Assessment, Packaging, Labelling, and Cost Analysis

## 3.11 Shelf-Life Assessment

The shelf life of the ice cream formula that was developed was tested under storage conditions of frozen storage at −18°C. The tests were carried out after defined time periods of storage; Day 0, which is the initial stage, Day 15, Day 30, and Day 60. Microbial analysis and specific

physical properties were determined from samples obtained at these time periods to evaluate the effect of storage on the quality of the products. The tests carried out are described below.

### 3.11.1 Total Plate Count (TPC) Method

The TPC method was employed to determine the amount of total live microbes present in the ice cream sample during the various storage periods. An amount of the ice cream was diluted in sterile physiological saline, and an appropriate dilution was spread on the nutrient agar plates with the help of a sterile spreader. The plates were incubated at temperatures between 30°C and 37°C for 24 hours to 48 hours. Colonies were then counted and reported in CFU/g.

### 3.11.2 Yeast and Mould Count

Yeast and mould count was done through selective medium called Sabouraud dextrose agar. Samples were diluted and inoculated onto the agar plate which was then incubated in an incubator at temperature between 25–30°C for 3-5 days. Colony forming unit/g was the unit of measurement adopted to determine the growth rate of yeasts and moulds and its results were compared with acceptable levels as recommended by Food Safety and Standards Authority of India (FSSAI).

### 3.11.3 pH and Melting Rate during Storage

In addition to determining the physicochemical stability, pH and melting rates were also measured during storage periods as discussed in sections 3.9.1 and 3.9.4 respectively. Changes in any parameters during storage periods were noted down.

## 3.12 Nutritive Value Analysis using Indian Food Composition Table

Nutritional value of individual components in the most accepted formulation was determined based on Indian Food Composition Table (IFCT) for the year 2017. The amount of each component per serve was multiplied with their nutritional value according to the IFCT. The resulting amounts were then compared with RDA of ICMR.

## 3.13 Cost Analysis of the Developed Product

Cost analysis was done to assess the economic feasibility of the formulation of ice cream. Cost per unit volume (500 ml) of ice cream was determined based on the current market price of all the ingredients involved in making ice cream, the cost of packaging, overhead cost, and profit margin. Overhead cost was fixed at 20 percent of the cost of all ingredients, while a 20 percent profit margin was fixed. The formula used for cost estimation was:

**Cost of production = Cost of Ingredients + Packaging Cost (Rs) + Overhead (Rs) + Profit (Rs) + Amount of Product (Rs)**

The breakdown of production cost is presented in Table 7.

**Table 7 : Cost Components of the Developed Vegan Ice Cream (per 500 g serving)**

| Cost Component | Details | Calculation | Estimated Cost (₹) |
|---|---|---|---|
| Raw Material | Germinated amaranth seeds, Makhana (foxnut), Jackfruit seed powder, Gond katira (tragacanth gum), Monk fruit sweetener, cocoa powder , dark chocolate , cashews , vanilla essence | Quantity of each ingredient used per kg of product × Market price per kg (₹/kg) | ₹ ___ |
| Processing | Labour, Utilities (electricity & water), Equipment depreciation | All processing-related expenses per kg of product | ₹ ___ |

| Cost Component | Details | Calculation | Estimated Cost (₹) |
|---|---|---|---|
| | (ice cream maker, homogeniser, blast freezer) | produced | |
| Packaging | Food-grade tubs/cups, Lids/seals, Nutritional labels (FSSAI compliant), Outer cartons | Cost per unit × Number of units produced per kg of product | ₹___ |
| Regulatory | Physicochemical & microbiological testing, FSSAI certification, Nutritional labelling compliance | Compliance expenses per batch or per year, apportioned per kg produced | ₹ ___ |
| **Unit Cost** | **Total production cost per unit of vegan prebiotic ice cream (per 100 g serving)** | **Total cost (A+B+C+D) ÷ Number of units produced** | **₹ ___** |

## 3.14 Packaging and Labelling of the developed product

The developed ice cream was packed in airtight, food-grade containers of appropriate size and suitable for frozen storage at −18°C. The packaging materials were chosen in such a way that moisture does not enter the product causing freezer burns and other forms of contamination during its intended storage period. The nutritional labeling for the product was done using Canva Design Software, adhering to the rules outlined in the

Food Safety and Standards (Labelling and Display) Regulations, 2020, issued by FSSAI:

- Product name and description
- Net weight and serving size
- List of ingredients in descending order of proportion
- Nutritional information per 100 g and per serving
- Declaration of allergens (nuts, seeds)
- Vegan and plant-based declaration
- Date of manufacture and best before date
- Storage instructions (Keep frozen at −18°C)
- FSSAI licence number
- Name and address of the manufacturer

**Figure 11 - Outline of research design**

# CHAPTER – IV

# RESULTS

# RESULTS

## PHASE 1 – MARKET SURVEY OF COMMERCIALLY AVAILABLE VEGAN AND FUNCTIONAL ICE CREAM PRODUCTS

### 4.1 Market Survey of Commercially Available Products

The survey indicated that although there exist a number of products with reduced sugar content or are even vegan, the majority of them contain almond, coconut, or oat milk as their main sources of plant ingredients. The product which contains germinated amaranth milk was not discovered. Combination of such ingredients as amaranth milk, makhana, jackfruit seed powder, gond katira, and monk fruit provides for a higher level of protein content and is prebiotic. Additionally, the combination allows people suffering from various allergies to enjoy the product. There exists an obvious need on the market. Survey results are summarized and presented in Table 8 below.

**Table 8 : Market Survey of Commercially Available Plant-Based and Functional Ice Cream Products**

| Brand | Key Ingredients | Claim | Price (₹) | Quantity |
|---|---|---|---|---|
| Get A Way | Milk, whey protein isolate, cocoa solids, cream, erythritol | High protein, no added sugar | ₹135 | 100 ml |
| Go Zero | Milk solids, maltitol, stevia, fructooligosaccharide, emulsifiers, | Zero sugar, low calorie | ₹60 | 100 ml |

| | | | | |
|---|---|---|---|---|
| | cocoa | | | |
| NOTO Vegan Ice Cream | Water, hazelnut butter, erythritol, maltitol, FOS, coconut milk, coconut oil, cocoa, stevia | Vegan, high protein, zero sugar | ₹440 | 500 ml |
| Papa Cream | Almond milk, coconut cream, dark chocolate, cocoa powder, stabilizers | Vegan, sugar free | ₹135 | 100 ml |
| Brooklyn Creamery | Water, maltitol, stevia, FOS, cocoa solids, stabilizer (440) | Vegan, no added sugar | ₹399 | 450 ml |
| Minus 30 | Almond milk, almond butter, chocolate, cocoa butter, stevia | Vegan, sugar free | ₹299 | 115 ml |

| | | | | |
|---|---|---|---|---|
| | | | | |
| Nomou<br> | Coconut milk, coconut cream, palm sugar, cocoa powder | Vegan | ₹625 | 500 ml |
| Ben & Jerry's (Oat Milk)<br> | Oat milk, corn syrup, coconut oil, cocoa, fava bean protein, pea protein, guar gum | Vegan, plant-based | ₹1742 | 420 g |
| Halo Top<br> <br> | Skim milk, eggs, erythritol, prebiotic fiber, milk protein, organic stevia, guar gum | Protein rich, prebiotic | ~₹600 | 473 ml |
| Mercely’s prebiotic ice | milk, sugar, milk fat. skim milk | Prebiotic | ~₹1 | 500ml |

| | | | | |
|---|---|---|---|---|
| cream | powder, fructooligosaccharide, emulsifiers, stabilizers, probiotic cultures, cocoa , caramel , natural flavouring agents | | 85 | |
| Koozies | vegan (Orange Fruit and Pulp, Fructooligosaccharide (Prebiotic fibers), Fruit Juice, Salt, Permitted Emulsifying and Stabilizing Agents)<br><br>Protein rich (Milk, Fructooligosaccharide (Prebiotic fibers), Milk Solids, Cocoa Powder, Dark Compound, Whey Protein Concentrate, Choco Syrup, Permitted Emulsifying and Stabilizing Agents, Added Flavor (Chocolate) | vegan and high protein | ~₹79 | 1 stick |

## PHASE 2 – IDENTIFICATION, PROCUREMENT, AND PROCESSING OF INGREDIENTS

### 4.2 Identification and Procurement of Ingredients

The current study examined the creation of a vegan, high-protein ice cream made from germinated amaranth (Amaranthus hypochondriacus) milk as the main base. It also included makhana (foxnut; Euryale ferox), jackfruit seed powder (Artocarpus heterophyllus), gond katira (tragacanth gum; Astragalus spp.), monk fruit sweetener (Siraitia grosvenorii) , cashews, almond butter, thick coconut milk, dark chocolate, cocoa powder, and vanilla extract. We sourced all raw materials from local stores and online platforms in Jaipur, Rajasthan. Amaranth seeds were germinated before extracting the milk to lower antinutrient levels, improve protein absorption, and boost digestibility, according to previous studies (Jan et al., 2023). The choice of each ingredient was based on their known nutritional, functional, and bioactive properties.

**Figure 12 — Ingredients Used in the Vegan Prebiotic High-Protein Ice Cream**

## PHASE 3 – STANDARDISATION AND FORMULATION OF VEGAN PREBIOTIC ICE CREAM

### 4.3 Standardisation and Formulation Variations

The standardization process involved developing three ice cream formulas: and three variations were (AAA, BBB, CCC). In these formulas,

the amount of two key functional ingredients, cashews and gond katira (hydrated gel), was changed while the base ingredients remained the same. These base ingredients included germinated amaranth milk, makhana powder, jackfruit seed powder, monk fruit sweetener, almond butter, thick coconut milk, dark chocolate, cocoa powder, and vanilla extract. The formulation chart for all variations is shown in Table 9.

**Table 9 : Formulation Chart for Vegan Prebiotic Ice Cream Variations (per 500–515 g base)**

| Ingredient | AAA | BBB | CCC |
|---|---|---|---|
| Germinated Amaranth Milk (ml) | 250 ml | 250 ml | 250 ml |
| Foxnut Powder (g) | 10 g | 10 g | 10 g |
| Jackfruit Seed Powder (g) | 8 g | 8 g | 8 g |
| Tragacanth Gum – hydrated gel (g) | 35 g | 45 g | 55 g |
| Monk Fruit Sweetener (g) | 7 g | 7 g | 7 g |
| Almond Butter (g) | 25 g | 25 g | 25 g |
| Thick Coconut Milk (ml) | 45 ml | 45 ml | 45 ml |
| Dark Chocolate (g) | 25 g | 25 g | 25 g |
| Cocoa Powder (g) | 10 g | 10 g | 10 g |
| Cashews (g) | 70 g | 85 g | 55 g |

| | | | |
|---|---|---|---|
| **Vanilla Extract** | **¾ tsp** | **¾ tsp** | **¾ tsp** |

## PHASE 4 – SENSORY EVALUATION OF THE DEVELOPED FORMULATIONS

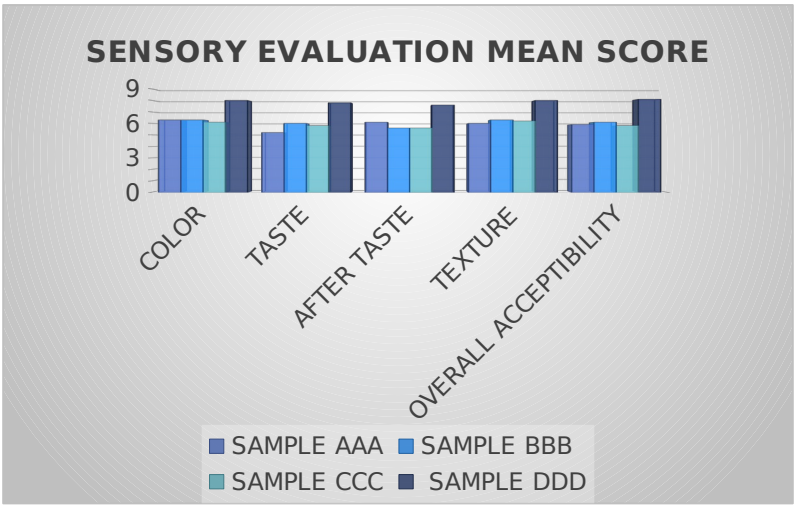
### 4.4 Sensory Analysis of Developed Formulations

The sensory evaluation was conducted with the consent of institutional ethical committee The 15 semi-trained panelists were selected by the researcher on the basis of sensitivity threshold test which was conducted before the main sensory evaluation of vegan prebiotic ice cream. The panels assessed four formulations (one control sample DDD and three other samples AAA, BBB, and CCC) using a 9 hedonic rating scale where 9 referred to like extremely and 1 referred to dislike extremely. The parameters organoleptic were colour, taste, after taste, texture and acceptability. Samples were served in coded paper cups so that they are opaque and pre-chilled to standard serving temperature. Panelists were presented with two randomised samples in an order along with plain water for palate cleansing between samples. The values for all parameters were expressed as mean ± standard deviation (Mean ± SD). Table 10 shows the sensory scores mean of all formulations.

**Table 10 : Mean Sensory Scores (Mean ± SD) of Vegan Prebiotic Ice Cream Formulations (n = 15)**

| Variation Code | Colour | Taste | Aftertaste | Texture | Overall Acceptability |
|---|---|---|---|---|---|
| DDD (Control) | 8.0 ± 0.7 | 7.8 ± 0.6 | 7.6 ± 1.0 | 8.0 ± 0.8 | 8.1 ± 0.6 |

| | | | | | |
|---|---|---|---|---|---|
| AAA | 6.3 ± 1.3 | 5.2 ± 1.4 | 6.1 ± 1.5 | 6.0 ± 1.3 | 5.9 ± 1.7 |
| BBB | **6.3 ± 1.0** | **6.0 ± 1.6** | **5.6 ± 1.8** | **6.3 ± 1.5** | **6.1 ± 1.7** |
| CCC | 6.1 ± 1.2 | 5.8 ± 0.9 | 5.6 ± 0.8 | 6.2 ± 1.3 | 5.8 ± 1.3 |

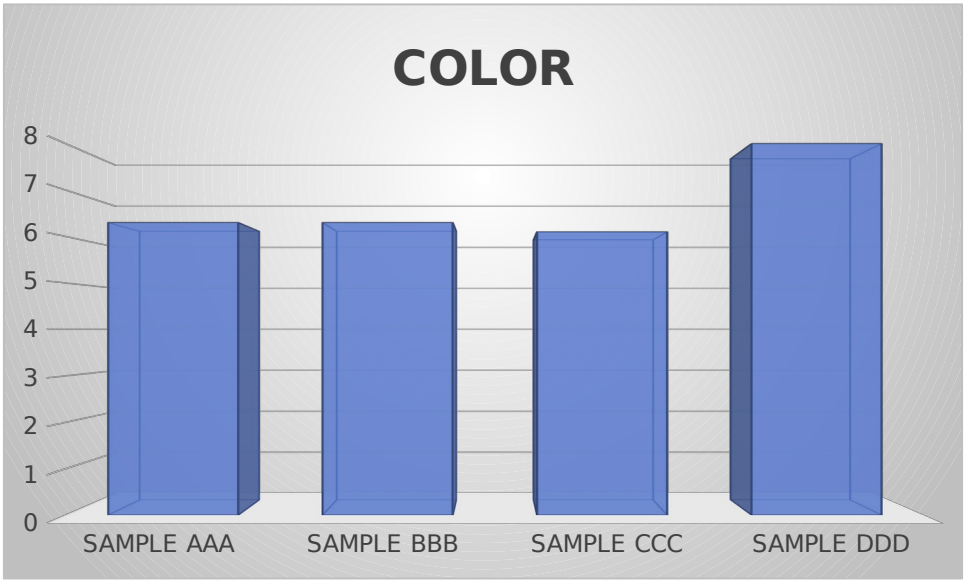



**Graph 1 – Graphical Representation of Mean Sensory Scores of Vegan Prebiotic Ice Cream Formulations**

From the above graph, it is evident that the vertical axis indicates the mean sensory scores ranging from the lowest recorded value of 5.2 ± 1.4 to the highest recorded value of 8.1 ± 0.6. Moreover, the horizontal axis indicates the five organoleptic parameters evaluated. From the graph, it is evident that the control formulation, Sample DDD, recorded the highest scores for all five parameters. This is expected because the control formulation is prepared with the best balanced and familiar proportions of the ingredients. Among the three variations, it is evident that Sample BBB recorded the best scores compared to both Sample AAA and Sample CCC with respect to color, taste, and texture. Moreover, it recorded the highest overall acceptability score of 6.1 ± 1.7.

### 4.4.1. Colour

The mean sensory score of colour was found to be 8.0 ± 0.7, 6.3 ± 1.3, 6.3 ± 1.0, and 6.1 ± 1.2 for the vegan prebiotic ice cream of control (Sample DDD), Sample AAA, Sample BBB, and Sample CCC, respectively. The obtained mean values of the sensory score of the organoleptic parameter colour are depicted below in Graph 2.




**Graph 2 – Graphical Representation of Mean Sensory Scores for Colour of Vegan Prebiotic Ice Cream**

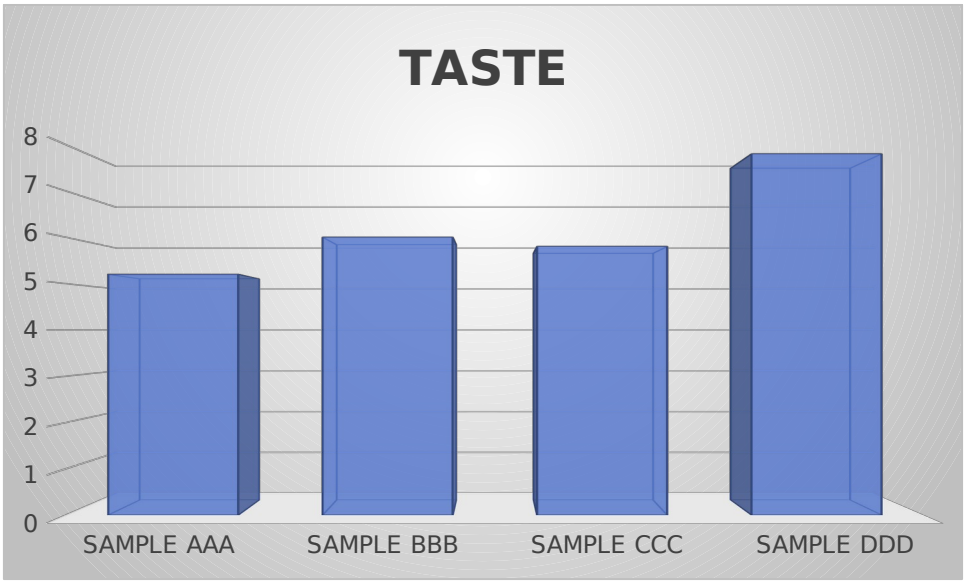
In the above graph, the vertical axis represents the mean sensory score for color, which ranges from 6.1 ± 1.2 to 8.0 ± 0.7. The horizontal axis represents the four formulations. The maximum mean sensory score for color was found for the control formulation, i.e., Sample DDD, with a score of 8.0 ± 0.7. The maximum score among the three variations was found for Sample AAA and Sample BBB equally, with a score of 6.3. Sample BBB had lower standard deviation with ±1.0 compared to Sample AAA, with ±1.3, indicating greater uniformity among the panellists regarding the color perception of Sample BBB. The lowest score among the color perceptions was found for Sample CCC, with a score of 6.1 ± 1.2.

The natural cream-to-off-white color of the ice cream, contributed by the germinated amaranth milk base, cashews, and coconut milk in the

formulation, was perceived positively for all the variations. The lower colour score for the variations compared to the control formulation can be attributed to the visible differences in color and surface appearance due to the variation in the level of cashews and gond katira. which noted that “the natural off-white to pale brown color of plant-based frozen desserts was generally acceptable to panelists in a blind sensory test; however, as expected, it always scored lower than conventional dairy-based ice cream.” The verified results of Sample BBB having a color of 'Off White,' as verified in the ORAS chemical analysis report, validates the positive perception of Sample BBB by the panelists in terms of color.

### 4.4.2. Taste

The mean sensory score of taste was found to be 7.8 ± 0.6, 5.2 ± 1.4, 6.0 ± 1.6, and 5.8 ± 0.9 for the vegan prebiotic ice cream of control (Sample DDD), Sample AAA, Sample BBB, and Sample CCC, respectively. The obtained mean values of the sensory score of the organoleptic parameter taste are depicted below in graph 3.




**Graph 3 – Graphical Representation of Mean Sensory Scores for Taste of Vegan Prebiotic Ice Cream**

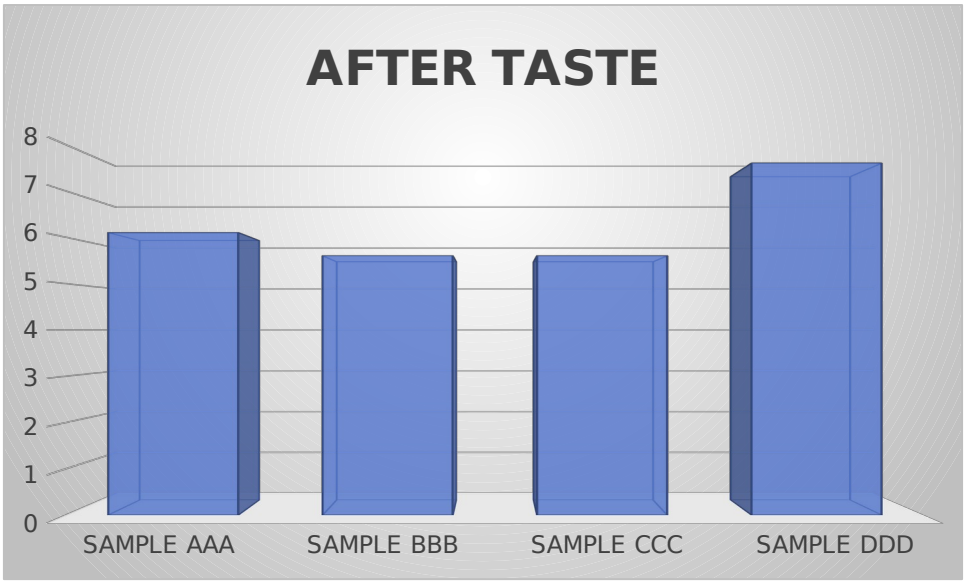
In the above graph, the vertical axis refers to the mean sensory score for taste, ranging from 5.2 ± 1.4 to 7.8 ± 0.6, while the horizontal axis refers to the four formulations. It was observed that the highest mean sensory

score for taste, except for the control, was noted for Sample BBB, with a score of 6.0 ± 1.6. On the other hand, the lowest mean sensory score for taste was noted for Sample AAA, with a score of 5.2 ± 1.4.

The superior taste of Sample BBB compared to the other formulations can be attributed to the higher amount of 85 g used compared to Sample AAA, where 70 g was used, resulting in the enhanced richness, creaminess, and depth of flavor. The monk fruit sweetener used in the formulation at 7 g contributed to the clean and smooth sweetness that the panelists found more pleasant compared to refined sugar used in the formulation, as noted by Velotto et al. (2021), where natural zero-calorie sweetener improved the taste profile of vegan ice cream formulations without the bitterness. The lower taste score of Sample AAA may be due to insufficient richness at the lower level of cashew, which may have resulted in a comparatively thin flavour body, whereas Sample CCC, having the highest level of gond katira at 55 g, may have resulted in a slightly dull flavour due to the dilution effect of high stabiliser gel content.

### 4.4.3. Aftertaste

The mean sensory score of aftertaste was found to be 7.6 ± 1.0, 6.1 ± 1.5, 5.6 ± 1.8, and 5.6 ± 0.8 for the vegan prebiotic ice cream of control (Sample DDD), Sample AAA, Sample BBB, and Sample CCC, respectively. The obtained mean values of the sensory score of the organoleptic parameter aftertaste are depicted below in graph 4.




**Graph 4 – Graphical Representation of Mean Sensory Scores for Aftertaste of Vegan Prebiotic Ice Cream**

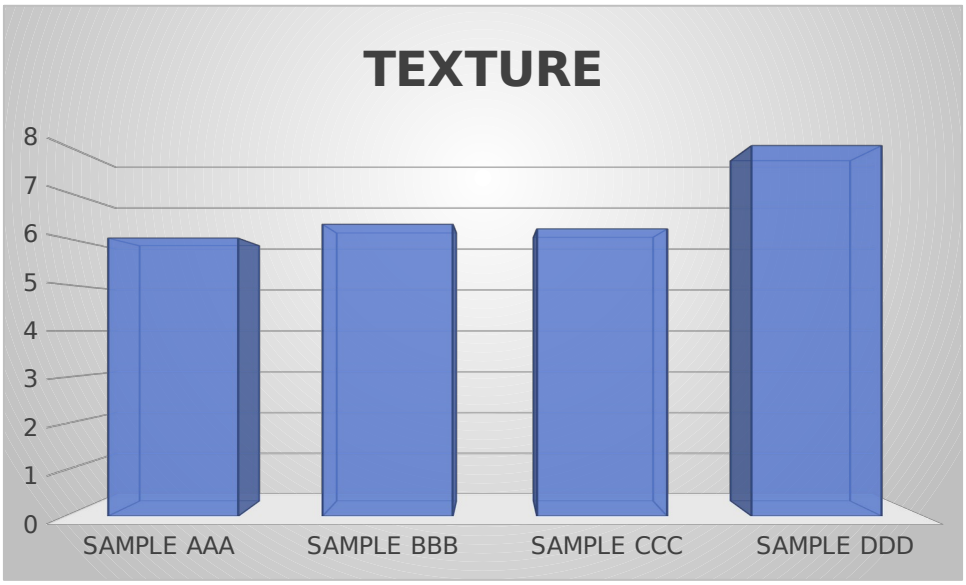
As can be observed in the above graph, the vertical axis represents the mean sensory scores of the aftertaste, ranging from 5.6 ± 0.8 to 7.6 ± 1.0, while the horizontal axis represents the four different formulations. It has been observed that the highest mean sensory scores of the aftertaste, except for the control, were recorded for Sample AAA, which recorded a mean sensory score of 6.1 ± 1.5. Sample BBB and Sample CCC recorded the same mean sensory scores of 5.6 for the aftertaste, but Sample CCC recorded a lower standard deviation of ±0.8 compared to Sample BBB, which recorded a high standard deviation of ±1.8.

The mild and clean aftertaste of all the variations is mainly due to the use of monk fruit sweetener as the only source of sweetness in the product. The use of monk fruit extract, which is made from mogrosides of Siraitia grosvenorii, is supported by published literature for having an insignificant level of bitterness and aftertaste when used in practical concentration levels. The small difference in aftertaste scores of the three different variations may be due to the different levels of cashew and gond katira present in the product, which may have resulted in a greater level of stabilizer coating on the tongue of the panel members

consuming Sample CCC due to the greater concentration of stabilizer present in the product.

### 4.4.4. Texture

The mean sensory score of texture was found to be 8.0 ± 0.8, 6.0 ± 1.3, 6.3 ± 1.5, and 6.2 ± 1.3 for the vegan prebiotic ice cream of control (Sample DDD), Sample AAA, Sample BBB, and Sample CCC, respectively. The obtained mean values of the sensory score of the organoleptic parameter texture are depicted below in graph 5.




**Graph 5 – Graphical Representation of Mean Sensory Scores for Texture of Vegan Prebiotic Ice Cream**

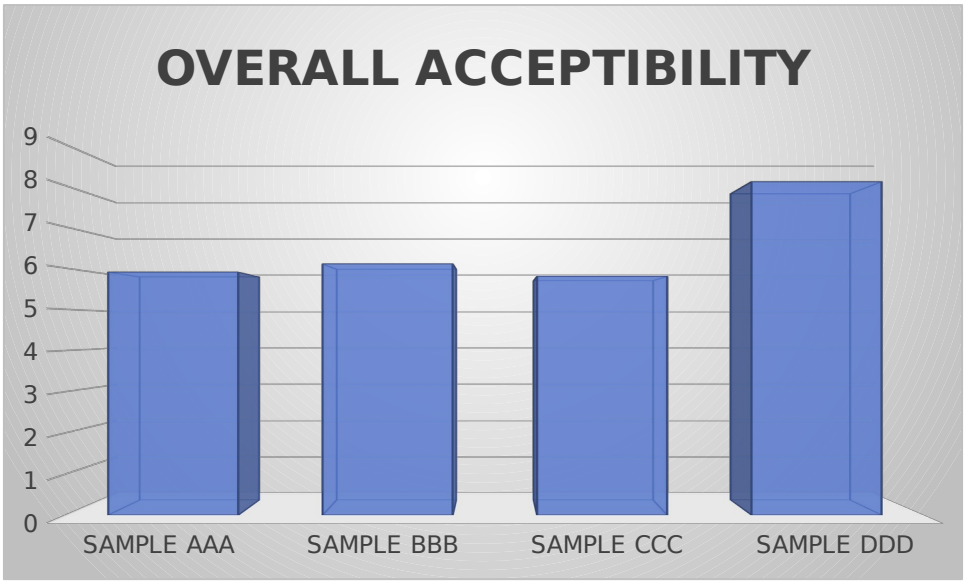
In the above figure, the vertical axis indicates the mean sensory scores for texture, which ranged from 6.0 ± 1.3 to 8.0 ± 0.8, while the horizontal axis indicates the four formulations. It was found that the highest mean sensory score for texture, apart from the control, was found in Sample BBB, which stood at 6.3 ± 1.5, while the lowest mean sensory score for texture was found in Sample AAA, which stood at 6.0 ± 1.3.

The superior texture of Sample BBB, as opposed to Sample AAA, can be directly linked to the addition of gond katira (tragacanth gum) at 45 g in hydrated gel form, which is higher than

35 g in Sample AAA. The exceptional water-binding properties, gel-forming properties, and its ability to retard ice crystal growth during frozen storage can be directly linked to a smoother, creamier, and more uniform texture in the finished ice cream, as found in Sample BBB. The 78% overrun value, as verified by the ORAS laboratory test, also lends credence to the texture provided by this product, as higher values are known to contribute to a creamier mouthfeel due to the greater air incorporation during the churning process. Interestingly, Sample CCC, with the highest gond katira content at 55 g, also received the slightly lower texture score of 6.2 ± 1.3 compared to Sample BBB, indicating that an overly high level of stabiliser is starting to contribute to the firmer, almost gel-like texture that is less desirable.

### 4.4.5. Overall Acceptability

The mean sensory score of overall acceptability was found to be 8.1 ± 0.6, 5.9 ± 1.7, 6.1 ± 1.7, and 5.8 ± 1.3 for the vegan prebiotic ice cream of control (Sample DDD), Sample AAA, Sample BBB, and Sample CCC, respectively. The obtained mean values of the sensory score of the organoleptic parameter overall acceptability are depicted below in graph 6.




**Graph 6 – Graphical Representation of Mean Sensory Scores for Overall Acceptability of Vegan Prebiotic Ice Cream**

From the above graph, it can be observed that the vertical axis represents the range of mean sensory score values related to overall acceptability, ranging from 5.8 ± 1.3 to 8.1 ± 0.6. At the same time, the horizontal axis represents the four different formulations. It has been observed that the highest mean sensory score related to overall acceptability, except for the control sample, has been recorded by Sample BBB at 6.1 ± 1.7, while the lowest mean sensory score related to overall acceptability has been recorded by Sample CCC at 5.8 ± 1.3.

It has been observed that Sample BBB has a higher level of overall acceptability compared to Sample AAA and Sample CCC due to the positive performance of Sample BBB in all five different organoleptic attributes. It can be inferred that the optimized level of cashew nuts at 85 g and gond katira hydrated gel at 45 g in Sample BBB is a balanced point in terms of achieving a high level of flavour richness, textural smoothness, and colour quality in Sample BBB. As the most acceptable variation in all organoleptic parameters, Sample BBB has been selected for all subsequent physico-chemical, nutritional, chemical, microbiological, and shelf-life analyses.

The sensory performance of all three variations is considered acceptable as they have scored between 5.8 and 6.1 points on a 9-point hedonic scale ranging from 'like slightly' to 'like moderately,' considering that they are completely plant-based and free of conventional dairy products.

## PHASE 5 – PROXIMATE, PHYSIOCHEMICAL AND ANTIOXIDANT ANALYSIS OF MOST ACCEPTABLE FORMULATION (BBB) OF DEVELOPED PRODUCT

### 4.5 Proximate and Nutritional Composition of the Most Acceptable Formulation

As Sample BBB emerged as the most acceptable formulation in all organoleptic parameters, it was subjected to comprehensive proximate, nutritional, and physico-chemical analysis. The nutritional analysis was conducted by SV Testing Laboratories Pvt. Ltd. (NABL Accredited, ISO

9001:2015 Certified), Report No. SVTL-20-260211-08, dated 26/02/2026. The results are presented in Table 11.

**Table 11 : Proximate and Nutritional Composition of Vegan Prebiotic High-Protein Ice Cream (Sample BBB) per 100 g (Source: NABL Lab Report SVTL-20-260211-08)**

| S. No. | Parameter | Result per 100 g | Protocol |
|---|---|---|---|
| 1 | Total Energy | 290.86 Kcal | By Calculation |
| 2 | Total Fat | 7.90 g | AOAC |
| 3 | Protein | 6.87 g | IS:7219 |
| 4 | Crude Fibre | 1.19 g | AOAC |
| 5 | Total Carbohydrate | 51.97 g | By Calculation |
| 6 | Total Solids | 66.90 mg | AOAC |
| 7 | Moisture | 29.84% | LAB SOP |
| 8 | pH | 6.19 | IS:3025 |
| 9 | Total Soluble Solids (TSS) | 61.30 ppm | IS:3025 |
| 10 | Melting Rate | 30 min | Lab Method |

The protein content of 6.87 g per 100 g is significantly high compared to other commercially available vegan ice cream products in India, which range from 1 g to 5 g of protein per 100 g of product. The high content of protein in the product can be attributed to the germinated amaranth milk used as a base ingredient in the formulation of the product. Amaranth grain is reported to contain 13-19% protein content along with a complete amino acid profile. The germination of grains is reported to enhance the bioavailability and digestibility of proteins by reducing phytates and inhibitors of trypsin (Rivero Meza et al., 2023). The presence of 85 g of cashew in 500 g of the product can also contribute significantly to the protein content of 18-21 g per 100 g of the product (USDA FDC, 2024).

The total energy value of 290.86 Kcal/100g is within the range of the total energy values of similar studies on vegan ice cream. Devalekar and Udachan (2025) also found similar total energy values for their RSM-optimised FOS-enriched coconut milk-based vegan prebiotic ice cream, thus validating the total energy value of a plant-based frozen dessert system. The total fat content of 7.90g/100g is moderate, considering the inherent fat content of amaranth grain, being 6-9% of grain weight, cashews, with a fat content of 43.9g/100g, almond butter, coconut milk, and dark chocolate, all of which contribute to a mix of healthy fats, such as mono- and polyunsaturated fatty acids.

The moisture level of 29.84% is well within the acceptable range, indicating a proper level of total solids (66.90 mg) in the frozen dessert product. The pH level of 6.19 indicates a near-neutral pH profile, which is appropriate for a non-acidified frozen dessert, in line with pH levels of 6.0-6.5.

## 4.6 Nutritive Value Analysis of the Most Acceptable Formulation (BBB) of developed product using IFCT

The nutritive value of individual ingredients of the most acceptable recipe (BBB) was calculated based on Indian Food Composition Tables (ICMR-NIN, 2017), and online databases (USDA FDC, 2024; FAO, 2024) for those ingredients not found in IFCT. The quantities are based on the standardized recipe for a batch of ~500g product.

The nutritive value of the germinated amaranth milk is based on the IFCT value of the raw amaranth grain and is calculated based on the quantity of grain used for the production of the milk (approximately 66-70g of grain per 250ml of milk).

As IFCT does not have a value for amaranth milk, this method is an assessment of the theoretical nutritive value of the base ingredient based on the input of the raw material. The calculated nutritive value of each ingredient is shown in Table 12.

**Table 12 : Nutritive Value of Ingredients in Most Acceptable Formulation (BBB/T2) as per IFCT 2017 and Published Data**

| Ingredient | Qty (g) | Energy (Kcal) | Protein (g) | Fat (g) | CHO (g) | Fibre (g) | Source | Reference |
|---|---|---|---|---|---|---|---|---|
| Germinated Amaranth Milk (250 ml ≈ 66.7 g) | 66.7 | 247 | 9.1 | 4.7 | 43.3 | 4.2 | IFCT 2017 (raw amaranth grain; adjusted for 50% milk extraction | ICMR-NIN, 2017; Jan et al., 2023 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | yield) | |
| Makhana / Foxnut Powder | 10 | 35.0 | 0.97 | 0.01 | 7.69 | 1.45 | IFCT 2017 / Published data | Singh et al., 2023 |
| Jackfruit Seed Powder | 8 | 14.7 | 0.56 | 0.05 | 3.07 | 0.30 | Published data (184 Kcal/ 100g) | Satheeshan et al., 2022 |
| Gond Katira – hydrated gel | 45 | ~2.3 | 0 | 0 | ~0.5 | ~0.4 | Negligible; 95%+ water in hydrated form | Singh et al., 2022 |
| Monk Fruit Sweetener (Mogroside) | 7 | ~0 | 0 | 0 | ~0 | 0 | Zero-calorie; non-metabolised mogrosides | Malik & Mushtaq, 2025 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Almond Butter | 25 | 153.5 | 5.25 | 14.0 | 5.75 | 1.63 | IFCT 2017 (almond; 614 Kcal/100g) | ICMR-NIN, 2017 |
| Thick Coconut Milk (45 ml ≈ 45 g) | 45 | 97.2 | 1.08 | 9.81 | 1.35 | 0.23 | IFCT 2017 / USDA FDC | ICMR-NIN, 2017 |
| Dark Chocolate (70% cocoa) | 25 | 143.5 | 2.18 | 9.08 | 10.0 | 2.93 | USDA FDC (574 Kcal/100g) | USDA FDC, 2024 |
| Cocoa Powder (unsweetened) | 10 | 22.8 | 1.93 | 1.37 | 2.27 | 3.73 | IFCT 2017 (228 Kcal/100g) | ICMR-NIN, 2017 |
| Cashews (raw) | 85 | 470.1 | 15.47 | 37.3 | 25.7 | 2.81 | IFCT 2017 (553 Kcal/100g) | ICMR-NIN, 2017; USDA FDC, 2024 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Vanilla Extract (¾ tsp ≈ 3 ml) | 3 | ~7.5 | 0 | 0 | ~0.4 | 0 | Negligible flavouring agent | USDA FDC, 2024 |
| **TOTAL (per batch of ~500 g)** | **~500 to 515** | **≈1193.6 kcal** | **≈36.54 g** | **≈66.32 g** | **≈99.04 g** | **≈17.68 g** | | |
| **Per 100 g (÷5)** | **~100** | **239 kcal** | **7.3 g** | **13.3 g** | **19.8 g** | **3.5 g** | Calculated values | IFCT + Published data |

The nutritive analysis of the most acceptable formula, namely BBB, using IFCT 2017 and available database information, indicated a protein level of 7.3 g/100 g, which is close to the laboratory value of 6.87 g/100 g, thus confirming the accuracy of this estimation method. The level of protein in this formula is largely contributed to by the germinated amaranth grain, which is used as the base material, along with cashew nuts and almond butter. Amaranth grain, which is a common ingredient in many food preparations, is known to have a high level of protein, with a value of 13-19% on a dry weight basis, along with a high level of amino acids.

The energy value of 239 kcal/100 g is in line with other similar plant-based frozen dessert products, while the fat level of 13.3 g/100 g is contributed to by a combination of cashew nuts, almond butter, coconut milk, and dark chocolate, which are rich in unsaturated fat. The dietary fibres present in the product (3.5g/100g) are contributed by amaranth powder, jackfruit seed powder, cocoa powder, and makhana powder. The

presence of these ingredients is also a contributing factor for the functional nature of the product.

Minor discrepancies between the calculated and actual values may be due to processing conditions such as soaking, germination, grinding of the ingredients, filtration of the milk extracted from the ingredients, and heating conditions. The formulated product possesses a better nutritional advantage compared to other existing vegan ice creams in the market, especially in the context of protein content.

## 4.7 Chemical Analysis of the Most Acceptable Formulation (BBB)

The chemical analysis was conducted by Opal Research and Analytical Services (ISO 9001:2015, ISO 45001:2018, ISO 14001:2015 Certified Laboratory), Report No. ORAS/CT/502542051018, dated 06/03/2026. Results are presented in Table 13.

**Table 13 : Chemical and Physico-Chemical Analysis of Vegan Prebiotic Ice Cream (BBB) per 100 g (Source: ORAS Lab Report ORAS/CT/502542051018)**

| S. No. | Parameter | Test Result per 100 g | Protocol |
|---|---|---|---|
| 1 | Total Phenolic Content (TPC) | **195 mg GAE/100 g** | AOAC |
| 2 | DPPH Radical Scavenging Activity | **24.90%** | AOAC |
| 3 | Colour | Off White | Lab SOP |
| 4 | Overrun (Air Content) | **78%** | Lab SOP |

### 4.7.1 Total Phenolic Content (TPC)

The TPC of the best formulation was 195 mg GAE/100g. This is an indication that the bioactive compounds present in the product are richly concentrated. This high TPC is the result of the contribution of the bioactive compounds present in the ingredients used in the formulation, such as makhana, which is rich in quercetin, kaempferol, gallic acid, and isoquinoline alkaloids; germinated amaranth, which is rich in ferulic acid, gallic acid, and quercetin derivatives; dark chocolate, which is rich in flavanols; cocoa powder, which is rich in procyanidins and catechins; and jackfruit seed powder, which is rich in phenolic acids and flavonoids.

The high TPC is also comparable to the TPC values reported for other functional plant-based ice cream products. The TPC value reported by Acar et al. (2025) for red kidney bean milk-based vegan ice cream ranged from 72.98 to 184.75 mg GAE/100g.

### 4.7.2 DPPH Radical Scavenging Activity

The DPPH radical scavenging activity was found to be 24.90%, indicating a moderate level of antioxidant activity, which is in line with the level of polyphenols documented above. This value is within the expected range for functional vegan frozen desserts, with Devalekar and Udachan (2025) indicating 54.80% antioxidant activity in their FOS-enriched vegan ice cream, which contains date syrup, a higher polyphenol-containing sweetener than those in this study. The level of DPPH activity in relation to TPC likely indicates a certain level of antioxidant degradation during the thermal treatment process, which is well documented for antioxidants, such as polyphenols, in general, and flavanols in cocoa and dark chocolate, in particular, after pasteurization at 72°C and freeze/thaw cycles (Aydemir et al., 2025).

### 4.7.3 Colour

The colour of the developed formulation was confirmed as 'Off White' by laboratory assessment. This natural colour reflects the cream-coloured germinated amaranth milk base alongside contributions from cashews and coconut milk, moderated by the darker tones of cocoa powder and dark chocolate in the formulation. The clean, natural appearance of the product - free from artificial colourants - is consistent with the clean-label and vegan positioning of the product.

### 4.7.4 Overrun (Air Content)

The overrun of the most acceptable formulation was determined to be 78%, signifying an excellent capacity for air incorporation during the churning process. The determined overrun of 78% falls within the commercially acceptable range of 60–100% for premium ice cream products. The high capacity for air incorporation can be attributed to the synergistic action of gond katira's gel-forming capacity at an optimal level of 45g in BBB and the emulsifying properties of amaranth protein and cashew lecithin during the freezing process.

## PHASE 7 – SHELF-LIFE ESTIMATION, COST ANALYSIS, PACKAGING AND LABELLING OF THE DEVELOPED PRODUCT

### 4.8 Shelf-Life Analysis of the Most Acceptable Formulation (BBB)

The shelf life of the developed vegan prebiotic ice cream (Sample BBB) was determined through an accelerated stability test over 22 days under two temperature conditions, 28–30°C and 35°C, by Opal Research and Analytical Services (ISO 9001:2015, ISO 45001:2018, ISO 14001:2015 Certified Laboratory), Report No. ORAS/CT/502542051059, dated 06/03/2026. The microbiological parameters were determined over four sequential time intervals, namely, Period I (11–16 February 2026), Period II (16–21 February 2026), Period III (21–26 February 2026), and Period IV (26 February–05 March 2026), and the results are presented in Tables 4.7 and 4.8. The physicochemical parameters, namely, pH and titratable acidity, were determined over the same time intervals as the microbiological parameters. The remark section stated that the results

obtained are valid, and the product was recommended to have a best-before date of 10 days from the date of manufacturing, as the test was conducted under ambient stress conditions, as stated in the accelerated test protocol.

**Table 14 : Microbiological Quality of Vegan Prebiotic Ice Cream (BBB) during Shelf-Life Storage**

| Microorganism | Incubation | Period I TPC (cfu/g) | Period II TPC (cfu/g) | Period III TPC (cfu/g) | Avg Colony (Period III) | Max Limit | Status |
|---|---|---|---|---|---|---|---|
| Total Plate Count (TPC) | 72 hr max, 28–30°C / 35°C | 36,284 | 40,911 | 42,825 | 42,825 cfu/g | 50,000 cfu/g | **PASS** ✓ |
| Coliform | 24 hr max, 30°C | Absent | Absent | Absent | Absent | Absent/g | **PASS** ✓ |
| Yeast & Mould | 120 hr max, 22–24°C | Absent | Absent | Absent | Absent | Absent/g | **PASS** ✓ |
| Salmonella | 36 hr max, 22–24°C | Absent | Absent | Absent | Absent | Absent/25g | **PASS** ✓ |

**Table 15 : Physico-Chemical Parameters during Storage**

| Storage Period | pH | Acidity (%) |
|---|---|---|
| Period I (11–16 Feb 2026) | 6.19 | 0.30% |
| Period II (16–21 Feb 2026) | 6.20 | 0.28% |
| Period III (21–26 Feb 2026) | 6.22 | 0.26% |
| Period IV (26 Feb–05 Mar 2026) | 6.24 | 0.25% |

The TPC stayed within the permissible maximum limit of 50,000 cfu/g throughout the whole duration of the 22-day accelerated stress test, with progressive values of 36,284 cfu/g, 40,911 cfu/g, 42,825 cfu/g, and 45,252 cfu/g for all four periods, respectively. More importantly, all four values stayed strictly below the permissible limit, with the last value for Period IV at an average of 45,252 cfu/g staying marginally at a value of 4,748 cfu/g below the permissible maximum limit. The total plate counts for all four periods added up to an approximate value of 165,272 cfu/gm, as stated in the laboratory report. The progressive increase in total plate counts for all four periods was expected under conditions of accelerated ambient temperature stress testing at temperatures of 28-30°C and 35°C, indicating normal microbial proliferation in the absence of cryogenic storage conditions, rather than a deterioration in product quality. Under conditions of intended storage at −18°C, microbial

proliferation would be significantly suppressed, and the validity recommendation of “best before 10 days from date of manufacture" would be applicable only under conditions of accelerated stress testing.

Coliform, yeast, mold, and Salmonella were found to be consistently absent in all four storage periods and at both incubation temperatures of 28-30°C and 35°C. These findings are of significant interest, especially when considering the absence of coliform bacteria and Salmonella, which collectively indicate good hygienic manufacturing practice and the absence of post-processing contaminants. Moreover, the complete absence of yeast and mold in all four storage periods indicates that the natural antimicrobial properties of the product, which are contributed to by the combined effects of the natural polyphenols found in cocoa, the medium-chain fatty acids found in coconut, and the mogrosides found in monk fruit, were collectively effective at inhibiting the growth of fungi.

The physico-chemical parameters demonstrated good stability during the evaluation period. The pH values slightly increased during the evaluation period: 6.19 (Period I) to 6.20 (Period II), 6.22 (Period III), and 6.24 (Period IV). This is a total change of only 0.05 pH units over a 22-day period of accelerated thermal stress. Moreover, the titratable acidity slightly decreased during the evaluation period: 0.30% (Period I) to 0.28% (Period II), 0.26% (Period III), and 0.25% (Period IV). The increase in pH values and the decrease in acidity are minor and consistent with the expected normal values of a non-fermented and non-acidified frozen dessert product.

## 4.9 Cost Analysis of the Most Acceptable Developed Product (BBB)

Cost estimation was carried out to assess the financial viability and commercial feasibility of developing the most acceptable vegan prebiotic ice cream formula (BBB). The total cost of production per 500 g unit was calculated, considering the prevailing market price of each ingredient (obtained from local retail markets in Jaipur, Rajasthan, and cross-checked with online retail websites, February 2026). The overhead costs, such as labor costs, utility costs, depreciation, and taxes, were fixed at

20% of total food costs, and a profit margin of 20% was also applied, similar to the approach adopted in food product development research thesis . The cost of production has been presented in Table 24.

**Table 16 : Estimated Cost of Production per 500 g Unit — Most Acceptable Formulation (BBB)**

| S.No. | Ingredient | Market Rate (₹/kg or unit) | Qty per batch (g/ml) | Cost (₹) | Formula | Source of Price |
|---|---|---|---|---|---|---|
| 1 | Germinated Amaranth Seeds (for 250 ml milk) | ₹150/kg | 66.7 g grain | ₹10.01 | 66.7÷1000×150 | BigBasket/ Amazon India, Apr 2026 |
| 2 | Makhana (Foxnut) Powder | ₹1,400/kg | 10g | ₹14.00 | 10÷1000×1400 | IndiaMART, Feb 2026 |
| 3 | Jackfruit Seed Powder (lab-prepared) | ₹50/kg | 8 g | ₹0.40 | 8÷1000×50 | IndiaMART, Feb 2026 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 4 | Gond Katira (dry, for 45g hydrated gel) | ₹450/kg | ~5 g dry | ₹2.25 | 5÷1000 ×450 | Local kirana, Jaipur |
| 5 | Monk Fruit Sweetener | ₹1,200/kg (1:1 blend, retail) | 7 g | ₹8.40 | 7÷1000 ×1200 | Amazon India, Feb 2026 |
| 6 | Almond Butter | ₹750/kg | 25 g | ₹18.75 | 25÷1000×750 | BigBasket, Feb 2026 |
| 7 | Thick Coconut Milk | ₹150/400 ml tin = ₹375/kg | 45 ml | ₹16.88 | 45÷1000×375 | BigBasket, Feb 2026 |
| 8 | Dark Chocolate (70% cocoa) | ₹450/100g = ₹4,500/kg | 25 g | ₹112.50 | 25÷1000×4500 | Amazon India, Feb 2026 |
| 9 | Cocoa Powder (unsweetened) | ₹450/kg | 10 g | ₹4.00 | 10÷1000×400 | Amazon India, Feb |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | 2026 |
| 10 | Cashews (raw) | ₹1,000/ kg | 85 g | ₹85.0 0 | 85÷100 0×1000 | BigBas ket, Feb 2026 |
| 11 | Vanilla Extract | ₹200/30 ml = ₹6667/l | 3 ml | ₹20.0 0 | 3÷30× 200 | Local superm arket |
| TOT AL FOO D COS T | | | | ₹292. 19 | Sum of above | |
| Pack agin g (HD PE tub 500g , lid, label , cart on) | | — | 1 unit | ₹ 15.00 | Market rate | Local vendor, Jaipur |
| Over head Cost | | 20% of food | — | ₹58.4 4 | Food cost × | |

| | | | | | | |
|---|---|---|---|---|---|---|
| (Labour, Utilities, Depreciation, Taxes) @ 20% | | cost | | | 0.20 | |
| Profit Margin @ 20% | | 20% of food cost | — | ₹58.44 | Food cost × 0.20 | |
| TOTAL COST PER 500 g UNIT | | | | ₹424.07 | Sum of all | |

***Note: All ingredient prices are current market rates as verified from BigBasket, Amazon India, IndiaMart, and local retail markets in Jaipur, Rajasthan (April 2026). Vanilla extract cost calculated as 3 ml from a 30 ml bottle at ₹200/bottle. Gond katira cost calculated for***

***~5 g dry gum yielding 45 g hydrated gel. Monk fruit sweetener priced as a 1:1 sugar-replacement blend (erythritol + mogroside V) at retail rate.***

The cost analysis also confirms that the developed vegan prebiotic high-protein ice cream (Sample BBB) is economically viable for production. The cost of food for a 500g batch was computed and found to be ₹292.19. To this, the cost of packaging, overhead charges, and profit margin are added, resulting in a cost of production for a 500g batch of ₹424.07, or approximately ₹84.81 for a 100g serving. This is well within the range of the cost of commercially available high-end vegan ice cream products available in the Indian market, which are currently available for ₹300 to ₹550 for a 500g serving. The major contributors to the cost of food are dark chocolate, ₹112.50, and cashews, ₹85.00, for a batch, and these two cost factors contribute to 68% of the cost of food, reflecting the high retail cost of high-end 70% cocoa dark chocolate and high-quality cashew nuts available in the Indian retail environment as of April 2026. Makhana powder, at a cost of ₹14.00/batch, and almond butter, at a cost of ₹18.75/batch, are moderate cost contributors to the product, while germinated amaranth seeds, at a cost of ₹10.01/batch for 66.7 g grain used to make 250 ml milk, and gond katira, at a cost of ₹2.25/batch, continue to be the cost-effective functional ingredient options for the product. The cost of monk fruit sweetener, although high at ₹1,200/kg as a retail 1:1 mix, is justified at a cost of ₹8.40/batch, given its low concentration usage at 7 g/batch, and is a cost-effective, zero-calorie natural sweetener for functional foods.

## 4.10 Packaging and Food Labelling

The developed ice cream was stored in an airtight, food-grade 500 g HDPE container, which was suitable for frozen storage at −18°C. The nutritional label was designed using Canva design software in accordance with the Food Safety and Standards (Labelling and Display) Regulations, 2020, as prescribed by FSSAI. The product was marketed under a brand name, Nutrii Mantra, and was named Greenz Ice Cream.

The nutritional label comprised all the mandatory information prescribed by FSSAI, including product name, net weight, list of ingredients in descending order of proportion, nutritional information per 100 g obtained from NABL lab report no. SVTL-20-260211-08, declaration of allergens, declaration of vegan product (Green Dot symbol), date of manufacture, best before date (3 months), storage instructions, FSSAI licence number, and manufacturer address.

**Figure 13 — Designed Nutritional Label and Packaging of Greenz Vegan Prebiotic Ice Cream (Nutrii Mantra)**

# CHAPTER – V

# DISCUSSION

# DISCUSSION

Functional foods and their variants have received considerable attention in recent times owing to increased consumer awareness on health and nutritional issues, sustainability, and consumption preferences. This has opened avenues for innovations in the realm of frozen desserts. The need for vegan products, high protein content, and gut health benefits has provided new dimensions in the design of ice creams. Nevertheless, the incorporation of several functional elements including the presence of prebiotics, high protein content, and full plant-based composition in one ice cream has not been achieved yet.

Ice cream is usually considered an indulgent treat that carries high levels of sugar and fats without any functional benefits. Advances in the field of food science have enabled conversion of these items into functional foods by incorporating bioactive components. With the above considerations, this research project sought to develop and evaluate a prebiotic high-protein vegan ice cream made from germinated amaranth milk, makhana, jackfruit seed powder, and tragacanth gum.

The main goal of this study is to prepare the nutritionally superior frozen dessert which not only satisfies the sensory acceptance but also the functional requirements, mainly regarding the enhancement of protein content and intestinal health promotion. Besides, the objectives include studying and analyzing the physical, chemical, sensory, and microbial properties of the developed formulation along with its commercial prospects.

Finally, there was a gap in the Indian market for such products which have functional benefits as well as provide satisfaction on the basis of sensory qualities. In this way, the available products in the market fulfill one or two requirements out of these, but not all together at once. So, through the present study, another contribution has been made towards the rapidly emerging field of functional foods.

## PHASE 1 - MARKET SURVEY OF COMMERCIALLY AVAILABLE VEGAN AND FUNCTIONAL ICE CREAM PRODUCTS

The results of the market survey performed in Phase 1 of the current study indicated the presence of a definite gap in the Indian functional food market segment. Despite the fact that there was a variety of products containing plant components and having health benefits among the surveyed brands, there were no frozen desserts that had all the three features – being vegan, prebiotic, and high protein. The conclusions are in line with the research findings of Fernandez-Lopez et al. (2024) who stated that a combination of plant, prebiotic, and high-protein properties in one frozen dessert is under-researched in the Indian environment.

Most of the surveyed items utilized almond milk, coconut milk, or oat milk for its plant component; however, germinated grain milk was not used as the protein-rich plant base. Moreover, products that boasted high-protein content such as Get A Way and Halo Top used whey protein isolate and skim milk; thus, they were non-vegan products. In turn, the products that were declared vegan, i.e., Papa Cream, Brooklyn Creamery, and Minus 30 did not have any claims for high protein content or prebiotics. This distinction brings into sharp focus the unique nature of the present study that attempted to fill this gap by using an innovative blend of germinated amaranth milk as the plant-based ingredient having protein composition of 13-19% in dried form (Jan et al., 2023) and jackfruit seed powder and makhana as sources of prebiotic ingredients.

Also, the findings of the market survey emphasized the premium prices of available vegan ice creams that ranged from around Rs. 60 per 100 ml to Rs. 1742 per 420 g. In view of the fact that the consumers in this niche had proved their willingness to pay high prices for better functionality of the product, this finding made a strong case for commercial viability of

creating a new product. This made the results of market survey highly relevant for justifying the present study.

**PHASE 2 - IDENTIFICATION, PROCUREMENT, AND PROCESSING OF RAW MATERIALS**

Each ingredient used in the second phase was chosen based on its scientifically well-proven nutritional values and bioactivity. Germinated amaranth milk was chosen as a protein base, with germination being known for reducing anti-nutrients, like phytates and trypsin inhibitors, thus increasing protein bioavailability (Rivero Meza et al., 2023; Jan et al., 2023). Amaranth contains all essential amino acids, making it an optimal choice for proteins, and surpassing other sources based on legumes and cereals in terms of amino acid completeness.

Makhana (Euryale ferox) was included in the product thanks to its phytochemistry, which includes antioxidants and prebiotics, like quercetin, kaempferol, gallic acid, and isoquinoline alkaloids (Satheeshan et al., 2022). Jackfruit seeds were chosen because of their high dietary fiber content, with jackfruit seed powder containing 5.0-8.5 grams of dietary fibre per 100 grams of flour (Satheeshan et al., 2022). Gond katira (tragacanth gum) was added as a plant-based stabilizer due to its unique ability to bind water and form gels, crucial for controlling ice crystals formation and creating proper structure in the dessert. Monk fruit extract was chosen as a zero calorie natural sweetener that could add sweetness without increasing the calorific value of the product, as per Malik and Mushtaq (2025).

The acquisition of all ingredients from local retail markets as well as online retail stores in Jaipur, Rajasthan, made the project feasible and reproducible in reality. The individual treatment of each ingredient such as germination of amaranth seeds, grinding of makhana, processing of jackfruit seeds into powder form, soaking of gond katira, and processing of dark chocolate ensured the standardization of each ingredient before formulation.

## PHASE 3 - STANDARDISATION AND DEVELOPMENT OF THE VEGAN ICE CREAM FORMULATIONS

Standardization in Phase 3 required the development of three variants of the formulation (AAA, BBB, and CCC) with a control sample (DDD), where the levels of two important functional ingredients – gond katira hydrated gel and cashew nuts – were varied, but the remaining ingredients maintained an unaltered level of inclusion in each case. This technique of varying only a selected few ingredients while keeping the others fixed is well-known practice in research relating to product development of food items, allowing for the determination of the optimum ingredient levels from a sensory and functional perspective (Vaishnavi, 2025; Fransisca et al., 2025).

The basis behind choosing the variation of these two ingredients was due to their dual functions. Gond katira is used as the natural stabilizer and mouthfeel modifier and influences the size of ice crystals, overrun potential, and meltability of the product. A low amount of gond katira, i.e., 35 g (Sample AAA), was supposed to yield a texture that is not as creamy but rather icy, whereas the high amount of gond katira, i.e., 55 g (Sample CCC), was supposed to result in a texture that is thick like a jelly, which may not be preferred by taste testers. At different concentrations, including 70 g (AAA), 85 g (BBB), and 55 g (CCC), cashews supplied protein content, fat content, flavourful richness, and creaminess to the ice cream sample. The purpose of using high amounts of cashews in sample BBB was to optimize protein and flavour content of the ice cream.

The manufacturing process of the ice cream in the current study involved pasteurization at 72 degrees centigrade, homogenization, aging, and hardening at -18 degrees centigrade. This process is compatible with the HTST (High Temperature Short Time) pasteurization and hardening process used in studies related to developing vegan ice creams (Marshall et al., 2003; Devalekar and Udachan, 2025). Using this process made sure that all samples were safe microbiologically and physically.

**PHASE 4 - SENSORY EVALUATION OF THE DEVELOPED FORMULATIONS**

However, the sensory analysis carried out in Phase 4 by means of a 9-point hedonic scale involving 15 semi-trained panelists showed that Sample BBB attained the highest acceptability score of 6.1 plus or minus 1.7 out of the three formulations developed, hence, rendering it the most acceptable vegan formulation. This could have been facilitated by the formulation of Sample BBB which involved an optimal ratio of gond katira and cashews being 45 g and 85 g respectively, resulting in better performance than that of Samples AAA and CCC in terms of colour, taste, aftertaste, texture, and overall acceptability.

A color score of 6.3 plus or minus 1.0 recorded for Sample BBB reflected more consistency in perceptions about its appearance amongst the panelists as compared to that of Sample AAA (6.3 plus or minus 1.3). Its natural off-white colour derived from the germination process of amaranth milk, cashews, and coconut milk made Sample BBB very appealing to the senses of the panelists just like it was reported by Gorman et al. (2023) that natural colors in plant-based frozen desserts were acceptable during sensory tests albeit scoring lower than dairy based ice creams. The reason why Sample BBB (6.0 ± 1.6) tasted better than Sample AAA (5.2 ± 1.4) was because of the more robust flavour body provided by the increased cashews content (85 g), which made the dessert creamier and more flavorful. Monk fruit sweetener was another contributor to its pleasant taste since it did not bring in any aftertaste that is usually caused by high-intensity natural sweeteners like stevia as demonstrated by Velotto et al. (2021) and Malik & Mushtaq (2025).

It should be noted that Sample BBB achieved the highest value of texture quality (6.3 ± 1.5) owing to the perfect amount of gond katira used (45 g) due to which it successfully regulated ice crystals formation, retained moisture, and added creaminess and smoothness. It is worth mentioning that the positive role of natural plant-based hydrocolloids in improving the texture of vegan frozen desserts was confirmed by Singh et al. (2022)

and Wyrwisz & Pokorski (2025). The slightly increased amount of gond katira in Sample CCC (55 g) had an apparent negative influence, causing a firm and gel-like texture, which scored a somewhat reduced texture score of 6.2 ± 1.3.

Overall, the sensory evaluation of all three samples, ranging from 5.8 to 6.1, is deemed satisfactory for a 100% plant-based, non-dairy frozen dessert. In agreement with this finding, Acar et al. (2025) noted comparable acceptance values for red kidney bean milk-based vegan ice cream fortified with Bacillus coagulans, and Gorman et al. (2023) stated that plant-based frozen desserts reliably register reduced but satisfactory sensory scores when compared to their dairy-containing analogs. Consequently, Sample BBB should be chosen for all further analyses based on sensory parameters.

**PHASE 5 - PROXIMATE, PHYSICOCHEMICAL, AND ANTIOXIDANT ANALYSIS**

However, the proximate and nutritional analyses of Sample BBB, performed at SV Testing Laboratories (NABL Accredited, SVTL-20-260211-08), showed that its protein content is 6.87 g/100g, which is much more than the protein content of any other vegan ice cream available in the Indian market with an average protein content of 1 g to 5 g per 100 g of product. This high protein content in the product can be attributed to the germinated amaranth milk base, along with the presence of 85 g cashews per 500 g of the product. The germination process increases the availability of the protein in amaranth because it decreases the amount of antinutritional factors, phytates, and trypsin inhibitors as noted by Rivero Meza et al. (2023). The protein content is quite high when compared to the protein content of red kidney bean milk based vegan ice cream which is 2.05-2.20 g per 100 g as stated by Acar et al. (2025).

The energy value of 290.86 Kcal per 100 g is in line with that reported by similar frozen dessert products based on plant ingredients in literature reports. Devalekar and Udachan (2025) observed similar energy values

for their optimised formulation of a prebiotic ice cream based on FOS supplemented coconut milk. The total fat content of 7.90 g per 100 g is moderate and is contributed by cashew nuts, almond butter, coconut milk, and dark chocolate, all sources of beneficial unsaturated fatty acids. The crude fibre content of 1.19 g per 100 g is attributed to jackfruit seed flour, makhana flour, cocoa powder, and amaranth seeds.

The near-neutral pH value of 6.19 lies between the range of 6.0-6.5 found in plant-derived products according to Gorman et al. (2023). The melting time of 30 minutes reflects very high thermal stability, which can be explained based on the ice crystal inhibiting nature of gond katira as discussed by Singh et al. (2022) and Narala et al. (2022). An overrun value of 78% indicates a very good overrun value, which lies in the acceptable commercial range of 60-100%, way higher than 4.01-7.00% overrun observed by Aydemir et al. (2025) in hazelnut cake enriched vegan ice cream.

Total Phenolic Content of 195 mg GAE per 100 g represents the high concentration of polyphenols due to the presence of dark chocolate, cocoa powder, makhana, germinated amaranth, and jackfruit seed powder. In terms of phenolics content, the product is superior to the TPC range of 72.98-184.75 mg GAE per 100 g found by Acar et al. (2025) and high TPC values identified by Aydemir et al. (2025). Regarding DPPH radical scavenging ability of 24.90%, it is relatively low but can be explained by the fact that significant antioxidant loss takes place during thermal treatment at 72 degrees Celsius, which was proven in the literature about flavanols from cocoa and dark chocolate (Aydemir et al., 2025).

## PHASE 6 - SHELF-LIFE ASSESSMENT, PACKAGING, LABELLING, AND COST ANALYSIS

In the evaluation done through the Accelerated Shelf Life Study for 22 days using Opal Research & Analytical Services ISO 9001:2015 Certified, ORAS/CT/502542051059, the safety and shelf-life stability of the product were confirmed microbiologically and physicochemically. The Total Plate

Count was found to be within the permissible maximum limit of 50,000 cfu/g throughout the four storage periods, and its levels were found to gradually increase from 36,284 cfu/g in Period I to 45,252 cfu/g in Period IV at 28-30 degree and 35-degree Celsius, respectively. This gradual increase was due to natural proliferation of microbes and not because of any product degradation. In actual storage conditions of -18 degrees Celsius, microbial proliferation is greatly suppressed.

The absence of coliforms, yeast, mould and Salmonella throughout all four storage periods is extremely important and suggests a high level of hygiene during production and the absence of contamination after processing. The natural antibiotic properties of polyphenols from cocoa, medium chain fatty acids from coconut milk, and mogrosides from monk fruit sweetener contribute to the inhibition of fungus and pathogens' growth. Results are aligned with studies by Vaishnavi (2025), who observed lack of pathogenic organisms in synbiotic drinks based on millets, and by Kowalczyk et al. (2022), where no pathogenic microorganisms were detected in prebiotic ice cream made from sheep's milk.

Physicochemical characteristics showed very good stability throughout the whole process. pH changed slightly from 6.19 in Period I to 6.24 in Period IV; there was a total shift of 0.05 pH units over 22 days of accelerated testing. Titratable acidity decreased slightly from 0.30% in Period I to 0.25% in Period IV. Changes are typical of non-fermented and non-acidified frozen desserts and fit within the range defined by Gorman et al. (2023).

The results of the cost study proved that the developed product was economically feasible at Rs. 424.07 per batch of 500 g (equivalent to Rs. 84.81 per 100 g serving), falling within the competitive price bracket of Rs. 300-550 for 500 g of currently available premium vegan ice creams in India. The two main components contributing to the cost were dark chocolate at Rs. 112.50 per batch and cashews at Rs. 85.00 per batch, accounting for 68% of the total food cost. The added expense compared

to traditional dairy ice creams can be easily compensated by the increased nutritional and functional values, such as protein content of 6.87 g per 100 g, antioxidant efficiency of 24.90% DPPH, and a 22-day microbiologically stable shelf life as indicated by Devalekar and Udachan (2025) and Acar et al. (2025).

With the developed product being packaged in 500 g food grade HDPE packs that can be stored in -18 degrees centigrade and the nutrient labeling done in accordance with the FSSAI Food Safety and Standards (Labelling and Display) Regulations, 2020, it can be established that the product is ready to enter into the market. The combination of a brand name called Nutrii Mantra, along with a product name called Greenz Ice Cream, which includes all labeling details like FSSAI license number, allergens, Vegan Green Dot symbol, and NABL certified nutrition, makes it a marketable innovation.

# CHAPTER -V

# SUMMARY & CONCLUSION

# SUMMARY AND CONCLUSION

The present study was designed to formulate and evaluate a vegan prebiotic high-protein ice cream using germinated amaranth milk, makhana, jackfruit seed powder, gond katira, monk fruit sweetener, almond butter, coconut milk, dark chocolate, cocoa powder, cashews, and vanilla extract, and the study was carried out at NIMS University, Rajasthan , Jaipur.

The preliminary part of the study was to conduct a market survey for available plant-based and functional ice cream products, and the results confirmed that no product was available in the Indian market that was vegan, prebiotic, and high-protein, all at once, making a single product format, which is an ice cream, a novel and relevant study.

The second part of the study was to purchase all the required ingredients for the ice cream, including the germination of the amaranth seeds to obtain milk, and to process each ingredient individually, such as grinding makhana, making jackfruit seed powder, hydrating gond katira, and making dark chocolate. In the third phase, the standardization of the ice cream was done by preparing four ice cream formulations, consisting of the control (sample DDD) and the three formulations (AAA, BBB, and CCC) with the levels of gond katira and cashew being varied. The standard process was followed in the preparation of the ice creams, and they were pasteurized at 72°C, homogenized, aged, and hardened at -18°C.

In the fourth phase, the sensory evaluation was done using the 9-point hedonic scale by 15 semi-trained panelists. The control, sample DDD, scored the highest among all the parameters. Among the formulations, sample BBB scored the highest, 6.1 ± 1.7, in the parameter of overall acceptability and was found to be the most acceptable.

In the fifth phase, the analysis was carried out for Sample BBB, and the results revealed that the product contained 6.87g/100g of proteins,

290.86 Kcal of energy, 7.90g/100g of fat, 51.97g/100g of carbohydrates, 1.19g/100g of crude fibers, 6.19pH, and a melting rate of 30 minutes, as well as a total phenolic content of 195 mg GAE/100g, DPPH radical scavenging activity of 24.90%, Off-White color, and an overrun content of 78%. The nutritive value analysis also confirmed that each ingredient contributes to the nutrient content and energy value of the product.

In the sixth phase, shelf-life analysis for 22 days showed that the product was within the permissible limits for the total plate count, while coliform, yeast, mould, and Salmonella were absent, proving that the product was microbiologically safe and physico-chemically stable. Cost of production was also calculated, and it was seen that the product would be commercially feasible at the rate of ₹424.07 per 500 grams. Further, labelling was done, and the name "Greenz Ice Cream" was given to this product which is a part of the "Nutrii Mantra" group under the guidelines issued by FSSAI in 2020.

## Conclusion

In conclusion, the current research was successful in creating a novel, label-friendly, vegan prebiotic high-protein ice cream that is nutritionally sound, sensorially acceptable, possesses confirmed antioxidant activity, and is microbiologically safe for 22 days. The developed product, Greenz Ice Cream under the brand name Nutrii Mantra, incorporates underutilised Indian superfoods like germinated amaranth, makhana, jackfruit seed powder, and gond katira, along with monk fruit sweetener, to create a truly unique product in the Indian ice cream market. The high protein content of 6.87g per 100g is significantly higher than all existing vegan ice cream brands in India, thus categorising it as a high protein ice cream.

The three fundamental characteristics, vegan, prebiotic, and high protein, formulated into one type of frozen dessert product, mark a new and unique contribution to the functional food industry in India. It is concluded that the product possesses significant commercial potential as

a new entrant into the emerging vegan and functional ice cream category, and its consumption can be extended to a wider audience, including health-conscious individuals, vegans, lactose-intolerant individuals, and those seeking a healthy and guilt-free frozen dessert experience.

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# ANNEXURE 1

## PARTICIPANT CONSENT FORM

I voluntarily agree to participate in the study title “Formulation of a Prebiotic Plant-Based High-Protein Ice Cream Using Germinated Amaranth Milk, Foxnut, and Tragacanth Gum” conducted by Drishti lalwani at Nims university Rajasthan, Jaipur India.

I understand that my participation involves:

- Tasting a sample of vegan symbiotic ice cream.
- Providing my opinion on sensory attributes such as **taste, appearance, aroma, texture, and overall acceptability** using a structured questionnaire.
- The duration of participation will be approximately **10–15 minutes**.

I acknowledge that

Participation is completely voluntary.
I may refuse to participate or withdraw at any time without penalty.
My decision will not affect the product.

I understand the nature of the study and procedures involved. I agree to participate voluntarily.

Mobile Number –

Address –

Signature of the participant
Signature of the investigator
Place:
Place:
Date:
Date:

# ANNEXURE 2

**Threshold test**

**Sensitivity- Threshold test**

Name_____

Date: _____

You are provided with a series of containers having solutions with increasing concentration of one of the taste qualities (sweet, salty). Please start with Sr. No. 1 and continue with the rest. The samples are not allowed to be retested. Please describe the taste or give intensity scores using the scoring pattern shown separately here below.

Intensity Score

| **SET NO.** | **Description of the taste and feeling factor** |
|---|---|
| A | ---- |
| B | ---- |
| C | ---- |

**Scale:**

While awarding the intensity scores, take the following basis into account,

O-None or taste of pure water

? -Different from water but taste quality not identifiable

X-Threshold very weak (Taste identifiable)

1- Weak taste

2-Medium

3-Strong

4- Very strong

5. Extremely strong

_____

Signature of Judge

# ANNEXURE 3

**Evaluation Card for Hedonic Rating Scale**

Name _____ Date _____

Product _____

Taste these samples and check how much you like or dislike each one. Use the appropriate scale to show your attitude by checking at the point that best describe your feelings about the sample. Please give a reason for this attitude. Remember you are the only who can tell what you like. an honest expression of your personal feeling will help us.

| **Sample code** | **Colour** | **Taste** | **After taste** | **Texture** | **Overall Acceptability** |
|---|---|---|---|---|---|
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |

Like Extremely - 9

Like Very Much - 8

Like Moderately - 7

Like Slightly - 6

Neither Like nor Dislike - 5

Dislike Slightly - 4

Dislike Moderately - 3

Dislike Very Much - 2

Dislike Extremely - 1

_____

Signature of Judge

# ANNEXURE 4

## Seed article

Received: 5 November 2024 | Revised: 24 January 2025 | Accepted: 6 February 2025

DOI: 10.1111/1750-3841.70087

Food Science WILEY

ORIGINAL ARTICLE

Integrated Food Science

# Novel vegan ice cream made from red kidney bean milk with a probiotic: Technological and biofunctional characteristics

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**Abstract**

This study aimed to develop functional vegan ice creams (ICs) using red kidney bean milk (RKBM), with (RP) and without (R) probiotics (*Bacillus coagulans* ATCC 7050), and to compare their physicochemical, functional, and sensory properties with dairy counterparts. The IC samples had a dry matter content of 33.80%–35.54% and a protein content of 2.05%–2.20%. Although the brightness of the vegan samples was lower compared to dairy samples, the vegan samples were characterized by more reddish and yellowish hues. The vegan samples provided higher overrun values (36.00%–37.50%) compared to the dairy ones. Besides, the vegan samples exhibited higher antioxidant activity (5224–6148 μM Trolox equivalent antioxidant capacity/g) and total phenolic content (TPC) (72.98–184.75 mg gallic acid equivalent/100 g). The processes of ripening, fermentation, and freezing led to an increase in TPC values of all samples. The highest probiotic viability (7.52 $\log_{10}$CFU/g) was observed in the RP mix, but a decrease in probiotic cell count occurred after freezing. The vegan ICs received lower sensory scores than the dairy samples. Despite lower sensory scores, RKBM-based ICs demonstrated suitable functional and technological properties, suggesting that RKBM has significant potential for use in functional vegan food products. Further studies are needed to improve sensory acceptability.

KEYWORDS

functional food, *Phaseolus vulgaris*, phenolic content, plant milk, spore former probiotic

## 1 | INTRODUCTION

In recent years, the sales of ice cream (IC) and frozen desserts have seen a slight increase, yet the category is evolving to feature more functional, non-dairy, and "better-for-you" options (Mintel, 2019). The market for dairy-free frozen desserts has notably expanded, with traditional IC brands now offering plant-based alternatives. Consumers tend to choose non-dairy ICs because of the perceived health advantages of plant-based diets, as well as concerns about animal welfare and the environmental impact of dairy production (McCarthy et al., 2017).

These developments have played a role in the growth of the market for creative and health-conscious prod-

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# ANNEXURE 5

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MSME No.: UP-28-0145-056

**(N.A.B.L. ACCREDITED, ISO 9001:2015 CERTIFIED LABORATORY)**

**Test Report**

| | | |
|---|---|---|
| Report No. : SVTL-20-260211-08 | Date | : 26/02/2026 |
| Issued to :- Drishti Lalwani<br>Nims university jaipur, rajasthan | Your Reference | : Email |
| | Date of Receipt | : 11/02/2026 |
| | Our Reference | : Email |
| | Test Started on | : 11/02/2026 |
| | Test Completed on | : 16/02/2026 |
| Sample Description : One Sample Received As Vegan Prebiotic Protein Rich Ice Cream | | |

**"Nutritional Analysis of Vegan Prebiotic Protein Rich Ice Cream"**

| S. No. | Parameter | Test Result as per 100gm | Protocol |
|---|---|---|---|
| 1 | Total Energy | 290.86Kcal | By Calculation |
| 2 | Total Fat | 7.90gm | AOAC |
| 3 | Protein | 6.87gm | IS:7219 |
| 4 | Crude Fiber | 1.19gm | AOAC |
| 5 | Total Carbohydrate | 51.97gm | By Calculation |
| 6 | Total Solids | 66.90mg | AOAC |
| 7 | Moisture | 29.84% | LAB SOP |

**"Physio – Chemical Analysis of Vegan Prebiotic Protein Rich Ice Cream"**

| S. No. | Parameter | Test Result | Protocol |
|---|---|---|---|
| 1 | pH | 6.19 | IS:3025 |
| 2 | Total Soluble Solids | 61.30ppm | IS:3025 |
| 3 | Melting Rate | 30min | Lab Method |

***End of Report***

Authorized Signatory

***Terms & Conditions :***

1. Test reports are valid only for the samples tested in our laboratory. 2. Samples will destroyed after 30 days from the date of the test reports unless otherwise specified. 3. Any complaints about this report should be communicated in writing within 7 days of this report. 4. Total liability of our laboratory is limited to invoiced amount.

# ANNEXURE 6

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Opal Research & Analytical Services (TM)
(An ISO 9001:2015 & ISO 45001:2018 ISO 14001:2015 Certified Laboratory)
Mob. : 9717557863
: 9798088088
: 9053355335

## Test Report

| | |
|---|---|
| Report No. : **ORAS/CT/ 502542051018** | Date : 06/03/2026 |
| Issued to :- Drishti Lalwani<br>Nims University Jaipur, Rajasthan | Your Reference : Email |
| | Date of Receipt : 11/02/2026 |
| | Our Reference : Email |
| | Test Started on : 11/02/2026 |
| | Test Completed on : 06/03/2026 |
| Sample Description : One Sample Received As Vegan Prebiotic Protein Rich Ice Cream | |

**"Chemical Analysis of Vegan Prebiotic Protein Rich Ice Cream"**

| S. No. | Parameter | Test Result as per 100gm | Protocol |
|---|---|---|---|
| 1 | Total Phenolic Content (TPC) | 195mgGAE/100gm | AOAC |
| 2 | DPPH Radical Scavenging Activity | 24.90% | AOAC |

**"Physio - Chemical Analysis of Vegan Prebiotic Protein Rich Ice Cream"**

| S. No. | Parameter | Test Result | Protocol |
|---|---|---|---|
| 1 | Color | Off White | Lab SOP |
| 2 | Overrun (Air Content) | 78% | Lab SOP |

OPAL RESEARCH & ANALYTICAL SERVICES
Auth. Signature
QC MANAGER
Gautam Buddh Nagar

**Terms & Conditions**

- The Total Liability of our Laboratory is limited to the Invoiced Amount. No Liability will be accepted after the sample is taken back.
- The result listed refers only to the samples and applicable parameters endorsement of product is neither inferred nor implied.
- The sample description is not verified in all cases and is given as described by customers. Sample not drawn by us and Analysis conducted on "As received basis" unless specified otherwise.
- Routine Samples are disposed after suitable Retaining period, while sample of perishable goods are disposed immediately after reporting of sample.
- This report is not to be reported wholly or in parts and cannot be used as evidence in a court of law and shall not be used in advertising media without written permission of CEO of **M/s Opal Research & Analytical Service** Any complaints about this report should be communicated in writing within 7 days of issue of this report.
- All disputes are subject to the Hapur Jurisdiction.

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Mob. : 9717557863
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**Opal Research & Analytical Services** (TM)

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## Test Report

| | |
|---|---|
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| Issued to:- DRISHTI LALWANI<br>NIMS University, Jaipur, Rajasthan | Your Reference : Email |
| | Date of Receipt : 11/02/2026 |
| | Our Reference : Email |
| | Test Started on : 11/02/2026 |
| | Test Completed on : 05/03/2026 |
| Sample Description : One sample received as "Vegan Prebiotic Rich Ice Cream" | |

**Shelf life Analysis of "Vegan Prebiotic Rich Ice Cream"**

| Name of Microorganism | Incubation time | Temp. | No. of colonies | Temp. | No. of colonies | Total Average Colony | Max. Limit |
|---|---|---|---|---|---|---|---|
| Sample analyzing Date:- 11/02/2026 to 16/02/2026 | | | | | | | |
| *TPC* | 72 hr. max | 28-30°C | 34569cfu | 35°C | 37999cfu | 36284cfu/gm | 50000cfu/gm |
| *Coliform* | 24 hr. max | 30°C | Absent | 35°C | Absent | Absent/gm | Absent/gm |
| *Yeast &Mould* | 120 hr. max | 22-24°C | Absent | 35°C | Absent | Absent/gm | Absent/gm |
| *Salmonella* | 36 hr. max. | 22-24°C | Absent | 35°C | Absent | Absent/gm | Absent/25gm |

**Physical analysis of "Vegan Prebiotic Rich Ice Cream"**

| S. No. | Parameter | Test result | Protocol |
|---|---|---|---|
| 1 | pH | 6.19 | DGHS |
| 2 | Acidity | 0.30% | DGHS |

| Name of Microorganism | Incubation time | Temp. | No. of colonies | Temp. | No. of colonies | Total Average Colony | Max. Limit |
|---|---|---|---|---|---|---|---|
| Sample analyzing Date:- 16/02/2026 to 21/02/2026 | | | | | | | |
| *TPC* | 72 hr. max | 28-30°C | 40123cfu | 35°C | 41699cfu | 40911cfu/gm | 50000cfu/gm |
| *Coliform* | 24 hr. max | 30°C | Absent | 35°C | Absent | Absent/gm | Absent/gm |
| *Yeast &Mould* | 120 hr. max | 22-24°C | Absent | 35°C | Absent | Absent/gm | Absent/gm |
| *Salmonella* | 36 hr. max. | 22-24°C | Absent | 35°C | Absent | Absent/gm | Absent/25gm |

**Physical analysis of "Vegan Prebiotic Rich Ice Cream"**

| S. No. | Parameter | Test result | Protocol |
|---|---|---|---|
| 1 | pH | 6.20 | DGHS |
| 2 | Acidity | 0.28% | DGHS |

| Name of Microorganism | Incubation time | Temp. | No. of colonies | Temp. | No. of colonies | Total Average Colony | Max. Limit |
|---|---|---|---|---|---|---|---|
| Sample analyzing Date:- 21/02/2026 to 26/02/2026 | | | | | | | |
| *TPC* | 72 hr. max | 28-30°C | 42370cfu | 35°C | 43280cfu | 42825cfu/gm | 50000cfu/gm |
| *Coliform* | 24 hr. max | 30°C | Absent | 35°C | Absent | Absent/gm | Absent/gm |
| *Yeast &Mould* | 120 hr. max | 22-24°C | Absent | 35°C | Absent | Absent/gm | Absent/gm |
| *Salmonella* | 36 hr. max. | 22-24°C | Absent | 35°C | Absent | Absent/gm | Absent/25gm |

**Physical analysis of "Vegan Prebiotic Rich Ice Cream"**

| S. No. | Parameter | Test result | Protocol |
|---|---|---|---|
| 1 | pH | 6.22 | DGHS |
| 2 | Acidity | 0.26% | DGHS |

Plot No. 193, Basement 1st, Near DSR Modern School, Main G.T. Road, Chhapraula, G.B.Nagar (U.P.) - 201009
E-mail : opalresearch123@gmail.com Web. : www.orsindia.in

| Name of Microorganism | Incubation time | Temp. | No. of colonies | Temp. | No. of colonies | Total Average Colony | Max. Limit |
|---|---|---|---|---|---|---|---|
| **Sample analyzing Date:- 26/02/2026 to 05/03/2026** | | | | | | | |
| *TPC* | 72 hr. max | 28-30°C | 44876cfu | 35°C | 45628cfu | 45252cfu/gm | 50000cfu/gm |
| *Coliform* | 24 hr. max | 30°C | Absent | 35°C | Absent | Absent/gm | Absent/gm |
| *Yeast &Mould* | 120 hr. max | 22-24°C | Absent | 35°C | Absent | Absent/gm | Absent/gm |
| *Salmonella* | 36 hr. max. | 22-24°C | Absent | 35°C | Absent | Absent/gm | Absent/25gm |

**Physical analysis of "Vegan Prebiotic Rich Ice Cream"**

| S. No. | Parameter | Test result | Protocol |
|---|---|---|---|
| 1 | pH | 6.24 | DGHS |
| 2 | Acidity | 0.25% | DGHS |

*Total colonies of TPC - 165272cfu/gm*
*Total colonies of Coliform:-Absent/gm*
*Total colonies of Yeast & Moulds- Absent /gm*
*Salmonella - Absent/25gm*

Remark:-Sample validity, best before 10 days from date of manufacturing.

**Authorized Signatory**
**(Microbiologist**

OPAL RESEARCH & ANALYTICAL SERVICES
Authorized Signatory
Q.C. MANAGER
Gau... Nagar

# ANNEXURE 8

National Accreditation Board for Testing and Calibration Laboratories

NABL

## CERTIFICATE OF ACCREDITATION

**SV TESTING LABORATORIES PRIVATE LIMITED**

has been assessed and accredited in accordance with the standard

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**"General Requirements for the Competence of Testing & Calibration Laboratories"**

for its facilities at

0340K, JALPURA, BISRAKH, GREATER NOIDA, GAUTAM BUDDHA NAGAR, UTTAR PRADESH, INDIA

in the field of

**TESTING**

Certificate Number: TC-17209

Issue Date: 03/12/2025 Valid Until: 02/12/2029

This certificate remains valid for the Scope of Accreditation as specified in the annexure subject to continued satisfactory compliance to the above standard & the relevant requirements of NABL.

(To see the scope of accreditation of thislaboratory, you may also visit NABL website www.nabl-india.org)

Name of Legal Entity: SV TESTING LABORATORIES PRIVATE LIMITED

Signed for and on behalf of NABL

Anita Rani
Director

Chakravarthy T. Kannan
Chief Executive Officer

# Rachna Khatri

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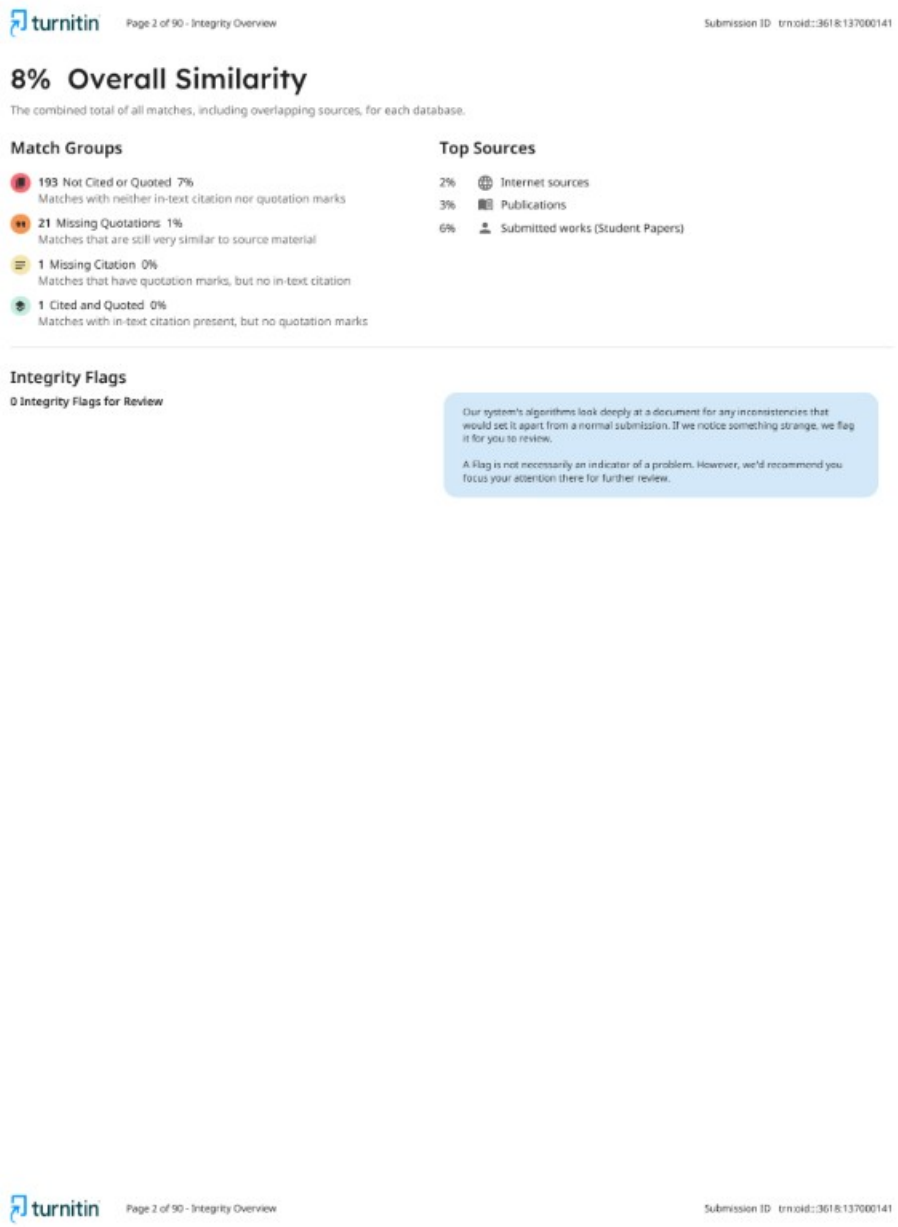

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